GENETICAL AND CYTOLOGICAL STUDIES

IN

THE GENUS P L A N T A G O.

THESIS

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I. INTRODUCTION

The totality of living organisms which comprises the plant kingdom presents an extreme diversity of form. In broad conception it may be said that the analysis and understanding of this diversity embraces the whole field of botanical study. Traditionally however plant classification has been the task of taxonomists using morphological data with ancillary considerations of geographical distribution and hybrid sterility. The primary concern of orthodox taxonomy is to supply recognizable reference points for the use of general biologists, although their classifications may be later the subject of theoretical, e.g. evolutionary, interpretation.

In recent years "experimental taxonomists" have developed a different method of approach to the problems of classification. This approach is based equally on the understanding of variation in the light of modern cytogenetic study and on intensive ecological and cultivation work with the living plants. The aim of this work is not limited by the practical considerations of orthodox taxonomy.

Thus Turesson (1922, 23, 25) showed that widely distributed species become differentiated into local

hereditary types, naturally selected by the action of local environmental factors on the gene complex of the plant population. The local populations become morphologically distinct, since some genes are selected, others being suppressed, by different combinations of habitat factors. The local populations may remain capable of interbreeding freely (ecotypes), though, with greater differentiation, interpopulation hybrids show increasing sterility. Such populations are termed "ecospecies". This type of analysis has been confirmed by numerous other workers.

Broadly speaking the categories of experimental taxonomy as at present visualised may be conveniently grouped under two heads:

(1) Infra-specific intra-fertile populations between which free gene exchange is potentially possible, though not often realised in nature, and

(2) Specific populations between which gene exchange is partially or totally prevented even when opportunity is presented.

The problem presented to the experimental taxonomists has two aspects; first to assess the degree of homology between the hereditary constitutions of different populations,and second to determine the gene exchange potential amongst them.

The present investigation deals with certain cytological problems which have been encountered during the course of an experimental taxonomic study of Plantago maritima L. and its allies. It has already been reported, Gregor (1939), that the N. American Greenlandic and N. European sea plantain populations are diploid (n = 6); those inhabiting the region of the Alps are of two kinds (a) diploid and (b) tetraploid (n = 12). Proof was obtained that the diploids and tetraploids can be crossed, Gregor (1939), but at that time it was not known whether gene transfer from one population to the other was actually possible. Consequently the two groups were provisionally regarded as belonging to separate coenospecies (i.e. to categories incapable of exchanging genes), but with the qualification that, "if it should be found that the diploid and tetraploid groups are capable of exchanging genes then they would belong to different ecospecies of the same coenospecies". It has therefore been the primary object of the present investigation to determine whether or not gene exchange between diploids and tetraploids has any foundation in fact and at the same time to assess the degree of relationship in and between all diploid and tetraploid populations.

That restricted gene exchange between populations is not necessarily expressed as visible morphological differentiation can be readily appreciated from the fact that a specimen supplied by the Royal Botanic Garden, Edinburgh, as Plantago alpina L. proved to be tetraploid, while other specimens collected from a reputed habitat of the same species have been found to be diploid. It might be argued that the morphological criterion of chromosome number would, alone, effectively solve this problem, but such a delimitation would, by itself, be quite inadequate as a means of recording the evolutionary independence of diploid and tetraploid populations. In such cases, therefore, taxonomic emphasis should be transferred from the morphological characteristics to the potential evolutionary significance of the populations concerned. A classification based on information of this kind would have a far greater theoretical significance, though obviously less practical utility, than one based on morphological criteria alone.

TABLE I.

Source of Material

WILD POPULATION SAMPLES

Ref. No.	Locality	Species	Collector
N. AMERICA			
Atlantic Coast.			
P82	New Haven, Conn.	P. oliganthos R.& S.	Dr. J.W.Gregor
P84	Gloucester, Mass.	P. juncoides Lam.	Mr. C.A.Weatherby
EUROPE			
Iceland.			
P104	Hlidarendi	P. maritima L.	Dr. J.W.Gregor
Alpine region.			
P63	Ostmark	P. serpentina All.	Dr. J.W.Gregor
P65	Ostmark	P. serpentina All.	Drs.Clausen & Gams.
P111	Allgäu	P. alpina L.	Dr. M.J.F.Gregor
P113	Allgäu	P. alpina L.	Dr. M.J.F.Gregor
P116(a)	Interlaken	P. alpina L.	Dr. R. Jenzer
P117	Geneva	P. alpina L.	Prof. F.Chodat
P117(a)	Geneva	P. alpina L.	Prof. F.Chodat
P118	Davos	P. alpina L.	Prof. A.Ernst
P118(a)	Davos	P. alpina L.	Prof. A.Ernst
P119	Fexthal	P. alpina L.	Dr. J.M.Cowan.

BOTANIC GARDEN SPECIMENS
(Received from the late Miss D. McCullagh).

P. alpina (Edinburgh Royal Botanic Garden)

P. carinata

P. serpentina

PsS9 = P. serpentina selfed.

II. MATERIAL AND METHODS.

Material. The material used in this investigation comprises types of European and N. American sea plantains allied to Plantago maritima L. The plants are in a collection maintained by Dr. J. W. Gregor and his colleagues at the Corstorphine Station of the Scottish Society for Research in Plant Breeding. The types in cultivation represent samples collected from wild populations, or received from botanic gardens, together with experimental hybrid lines. Dr. Gregor's work has comprised statistical studies of morphological characters, fertility and genetical behaviour, Gregor (1930, 38, 39).

The only cytological work previously carried out on the material was the determination of chromosome numbers in some samples by the late Miss D. McCullagh (vide Gregor), and by Miss E. S. Bennett (unpublished).

The types studied, with their sources, are listed in Table I.

A note on the terminology is requisite. The specific names attached to the various types are, so far as could be determined by the collector or Dr. Gregor in accordance with the orthodox taxonomy

of Plantago. Dr. Gregor has shown, however, that the characters used for distinction of species in this group are inconsistent, and consequently the specific names given in Table I (page 5) cannot be regarded as reliable. In this text the specimens of wild origin are referred to under their station numbers in contrast to those of botanic garden origin which retain the specific names under which they were received.

Methods. For the determination of the chromosome numbers root tips were fixed; they were taken from cuttings rooted in sphagnum moss but in a few instances from seedling plants potted in soil. Two fixatives were tried, Langlet's modification of Navashin's fluid, which had been found satisfactory for Plantago root tips by McCullagh (1934), and La Cour's (1931) 2 BE. The former was found reasonably satisfactory, 2 BE appeared to be useless. Following fixation overnight, the root tips were dehydrated and embedded in paraffin wax using La Cour's (1931) schedule. Sections were cut at 8 micron and stained by Newton's gentian violet-iodine technique.

Preparation of pollen-mother-cells at meiosis proved technically somewhat difficult. The smear technique was used extensively during the collecting seasons of 1938 and 1939. The particular method used

was to smear anthers between two slides, rapidly inverting one in a bath of the fixative 2 BE and immediately fixing and staining the smear on the other slide with iron aceto-carmine, Belling (1930). The aceto-carmine slide was available for immediate inspection to determine the stage of meiosis. If satisfactory this slide was made permanent by McClintock's (1929) method, while the smear fixed in 2 BE was stained with gentian violet and then mounted in Canada balsam. The method was persisted with because it was felt that a smeared film directly exposed to the fixative was likely to give the best fixation. Finally, however, the smear method was largely abandoned.

The size of the anthers varied considerably in different plants and, while the larger ones could often be smeared successfully, the smaller types only very rarely survived the pressure of smearing without ruinous crushing. Further the pollen-mother-cells in all types are firmly held together until just before first metaphase and very frequently an even smear of cells could not be obtained at stages before late first anaphase. Pollen-mother-cells closely adhering in groups frequently became badly crushed but even where they simply flattened under the cover slip, the nuclear details were not clearly observable. Added to these

technical difficulties were the facts that the first division of meiosis in this material appears to proceed very rapidly, so that at most two or three buds in an inflorescence would be at first metaphase together, and secondly that the flowering season of the plants was comparatively short (five to eight weeks according to the weather conditions). The preparation of permanent smears was therefore abandoned as a general method, because, although it occasionally gave good results it was too wasteful of time and material to allow preparations to be made of many different lines flowering together during a short season.

Fixation and subsequent paraffin embedding of the complete anther was substituted as a general method and aceto-carmine smears were made from only a few very favourable types. To determine the stage of meiosis in selected buds one anther was removed and crushed in aceto-carmine either before mounting or, in the case of very small anthers, by pressure or gentle heat after the cover slip was applied. The stage of meiosis could usually be determined from these preparations and if suitable, the remaining three anthers were dissected from the bud and fixed.

A variety of fixatives was tested:-
La Cour's (1931) 2 BE and 2 BD, Smith's (1935) S 1 and

S 2, *vide* Catcheside (1937), and medium Flemming, all of the osmic acid type, as well as Belling's (1930) Navaschin fluids, 1 and 2, and "Craf", Randolph (1935). It was hoped to find a suitable fixative containing osmic acid, since these swell the chromosomes less than the Navaschin type of fixatives. Belling's modification 2 was, however, finally adopted. It gave good chromosome fixation, usually with a clear non-reticulated cytoplasm and proved reasonably consistent. None of the fixatives containing osmium was at all consistent, although occasionally good results were obtained, notably with S 2 and sometimes with 2 BE.

The handling of samples of small anthers during dehydration and embedding presented difficulties as a routine operation. The normal procedure of dehydration in phials was useless because it required too much time and care merely to minimise the inevitable loss of the small anthers. The anthers after fixation were therefore washed out with water through a small funnel on to a piece of fine muslin attached round the base of the stem by a rubber ring. The material was then secured by tying the muslin into a small bag, which could be easily and rapidly transferred through the alcohol grades and into chloroform.

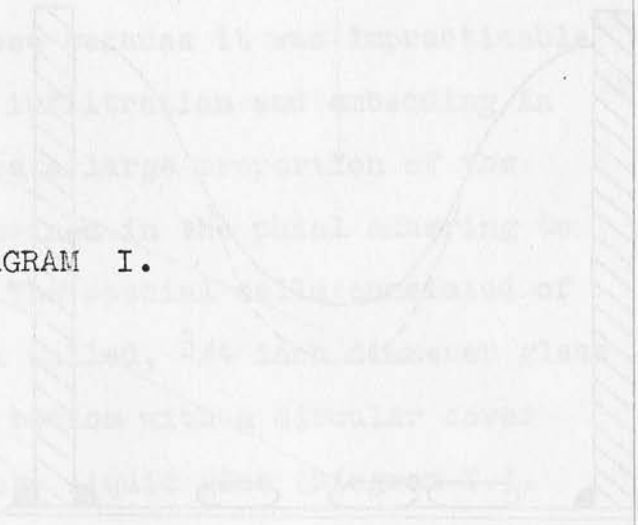


DIAGRAM I.

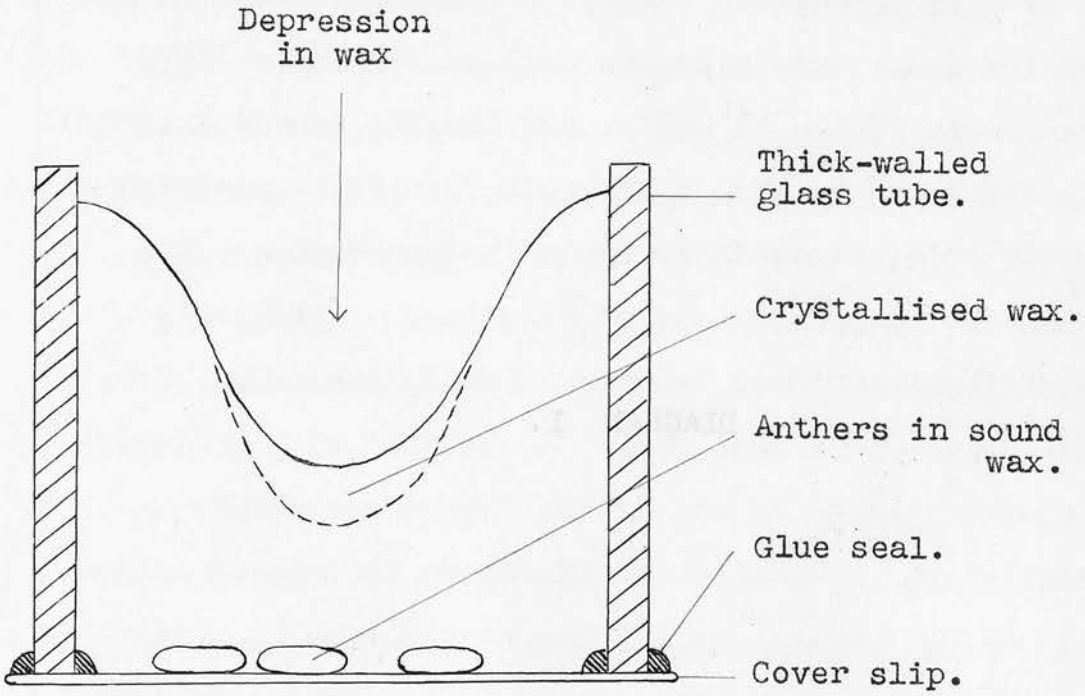


DIAGRAM I.

SECTION TO SHOW CONSTRUCTION

OF EMBEDDING CELL.

Having reached pure chloroform the anthers were removed from the muslin bags to special cells devised for infiltration and embedding. The need for a special cell arose because it was impracticable to carry out paraffin infiltration and embedding in separate vessels, since a large proportion of the anthers invariably remained in the phial adhering to the congealing wax. The special cells consisted of short lengths of thick walled, 3/4 inch diameter glass tubing, closed at the bottom with a circular cover slip using a proprietory liquid glue (Diagram I.). For infiltration of the wax these cells were placed in a stoppered wide mouthed jar and stood on top of the paraffin oven at 30°C. Later, inside the oven, the chloroform was evaporated and a wax mixture of melting point 50°C was added to the cells. The material was orientated with warm needles, whilst the cell stood on a ground glass plate warmed and illuminated from below by an electric bulb. The wax was then solidified by standing the cell in a shallow dish with cold water circulating around, but not over, the cell. The method of cooling the wax was found preferable to submergence in water immediately the surface film was formed. The difficulty with the normal procedure arose because the wax, contracting beneath its surface film, produced irregular internal cavities which

became filled with water through ruptures in the surface. With the cooling method adopted, the wax surface formed a deep cavity, crystallising a little at the bottom but invariably leaving underneath sufficient sound wax containing the material. The cells were left in water overnight when the coverslips either had soaked off or could be easily detached from the bottom and the wax block removed for trimming.

A summary of the schedule used in the preparation of the anthers is presented. In connection with the staining the best differentiation was obtained after rinsing in a saturated solution of picric acid in absolute alcohol as recommended by Smith (1934).

Preparations were also made of pollen grains with the object of measuring and comparing the size of the grains in different types. The pollen grains were mounted direct in Zirkle's (1937) gelatine-glucose aceto-carmine medium.

Schedule of Preparation for Anthers.

Fixation - 16-24 hours

Washing - 1 hour

Dehydration -

10% ethyl alcohol	-	½ hour
20% " "	-	½ hour
30% " "	-	1 hour
50% " "	-	2 hours
70% " "	-	overnight
80% " "	-	3 hours
95% " "	-	3 hours
absolute ethyl "	-	overnight

Transference to chloroform:-

3 parts	absolute alcohol,	1	part	chloroform	-	2 hours
2 "	" "	2	"	"	-	2 hours
1 "	" "	3	"	"	-	2 hours
pure chloroform						

Infiltration with wax.

The phials were placed on the top of the paraffin oven for 2-3 days and a piece of wax was added to the chloroform each day.

Embedding.

The phials were placed in oven at 52-3°C. Chloroform wax replaced by wax (MP 50°C) after 1 hour in the oven. Material embedded 2-3 hours later.

Sections cut at 14 microns.

Staining:-

0.5% aqueons gentian violet	- 10 mins.
1% iodine in 80% alcohol	- 45 secs.
95% alcohol	- rinse
saturated solution picric acid in absolute alcohol	- rinse
absolute alcohol	- 10 secs.
clove oil	- 10 secs.
3 changes in xylol during mount	- 15 secs.

Drawings. All drawings were made at bench level with a camera lucida, using a Watson 2mm. Holos. Immersion Objective and a 20x eye-piece with tube

TABLE II.

TABLE II.

TABLE II.

Chromosome numbers.

Population samples.	Chromosome No. mitosis	meiosis	Hybrids	Source	Chromosome No. mitosis	meiosis.
P21 *	12	-	C17	*P. serpentina* x P21	12	-
P63	24	12	C17(F_2)	F_2 population	12	-
P65	24	12	C21	*P. alpina* (Edin.) x PsS9	24	-
P81 *	12	-	C21(F_2)	F_2 population	24	12
P82	12	6	C22	*P. alpina* (Edin.) x P63	24	12
P84	12	6	C23	P63 x *P. alpina* (Edin.)	24	-
P91 *	12	-	C28	*P. carinata* x PsS9	12	6
P104	12	-	C28(F_2)	F_2 population	12 & 18	-
P111	12	-	C29	PsS9 x P84	12	6
P113	12	6	C32	*P. carinata* x P84	12	-
P116(a)	12	-	C37	*P. alpina* (Edin.) x P81	18	9
P117	24	-	C37 N.S.	Natural seed off C37	24	-
P117(a)	24	-	C38	*P. alpina* (Edin.) x P84	18	9
P118	12	-	C38 N.S.	Natural seed off C38	24	12
P118(a)	12	-	C39	*P. alpina* (Edin.) x P91	-	9
P119	12	-	C43	P113 x P104	12	-
P. alpina (Edin.)	24	12	C46	P113 x P65	24	-
P. carinata	12	6	C46 N.S.	Natural seed off C46	24	-
P. serpentina	12	6	C56	PsS9 x P113	12	-
PsS9	12	6				

* Determined by D. McCullagh.

length 160 mm., giving a magnification of 3100 diameters.

III. EXAMINATION OF MATERIAL

CHROMOSOME NUMBERS.

The chromosome numbers, determined at mitosis or meiosis or at both stages for all the samples and hybrids examined are enumerated in Table II. The basic number was uniformly x = 6; the population samples were either diploid with 2n = 12 or tetraploid with 2n = 24. No aberration from these figures was found in any of these plants.

The diploid chromosome number was found in hybrids between diploid parents and in their progeny. The only irregularity observed here was in an F_2 plant of the diploid hybrid C28, where one root tip showed eighteen chromosomes.

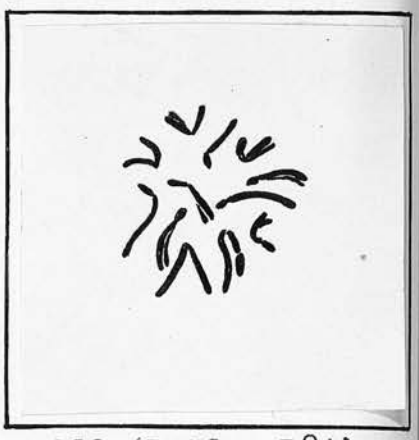
The triploid number was also observed in hybrids between tetraploids and diploids, notably C37, C38 and C39. In two cases, however, of tetraploid x diploid, the hybrids C21 and C46 were tetraploid. In two plants C37 N.S. and C38 N.S., the progeny respectively of the triploids C37 and C38, twenty-four

PLATE I.

PLATE I. MITOTIC CHROMOSOMES.

P113

P. carinata

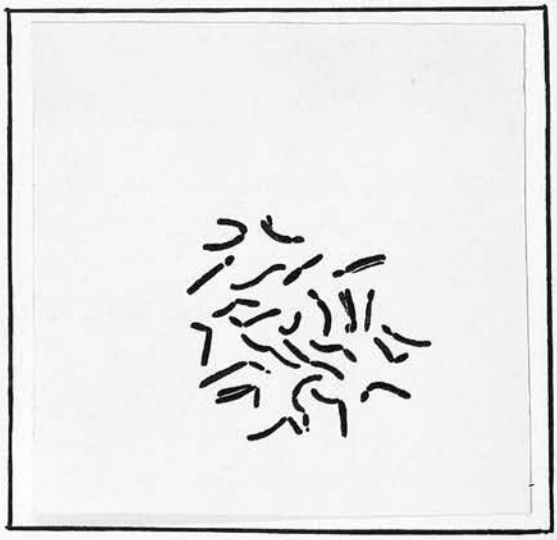


C29 (PsS9 x P84)

P84

C37 (P. alpina (Edin.) x P81)

P63



C21 (P. alpina (Edin.) x PsS9)

chromosomes were observed.

The remaining tetraploid hybrids resulted in accordance with expectation from crosses of tetraploid parents, or as progeny of such crosses.

MITOTIC CHROMOSOMES.

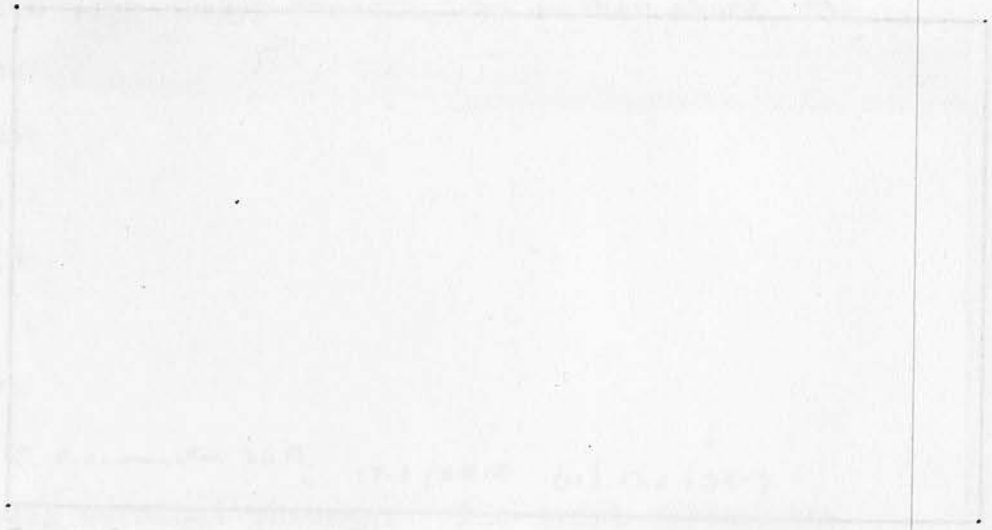
The chromosomes in all cases were similar in appearance being elongate and quite slender. The centromere "constriction" was usually clear, varying in different chromosomes from a median to a sub-terminal position.

In the diploid types a trabant or satellite was frequently present on two sub-terminally constricted chromosomes. Three such chromosomes were sometimes observed in triploids but more than two was not seen in any tetraploid. In all cases, however, the satellite was very small and its absence in some cells and types was probably due to local fixation effects.

A high degree of similarity was apparent amongst mitotic complements of all forms studied. No distinctions were possible save that of chromosome number.

A representative series of mitotic plates is illustrated in Plate I.

PLATE II.



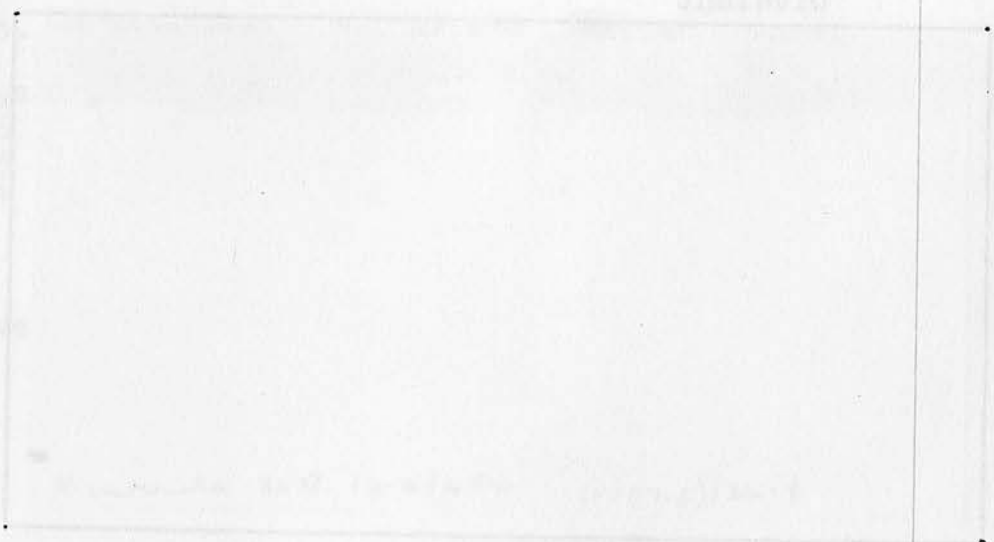


PLATE II. P. CARINATA.

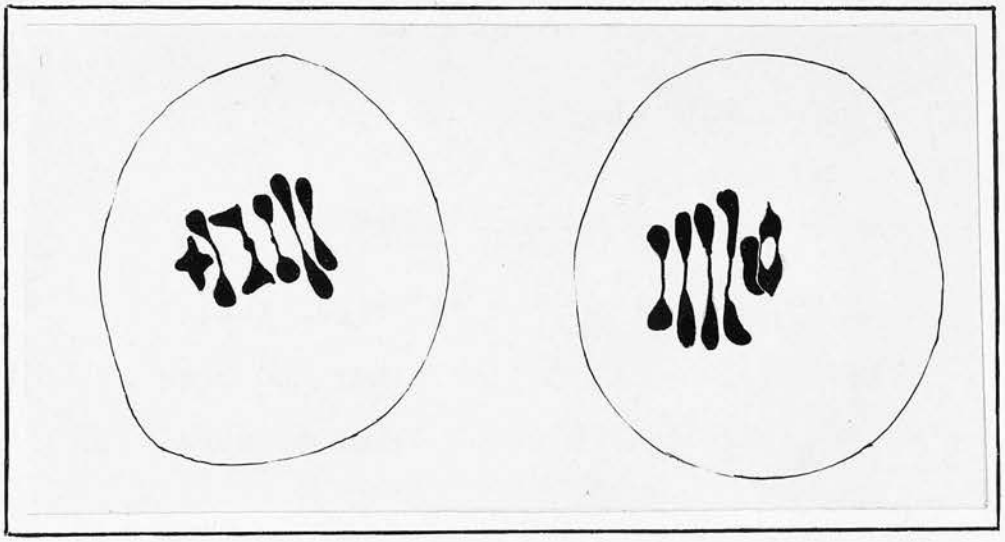


Fig. 1. Metaphase

Fig. 2. Metaphase

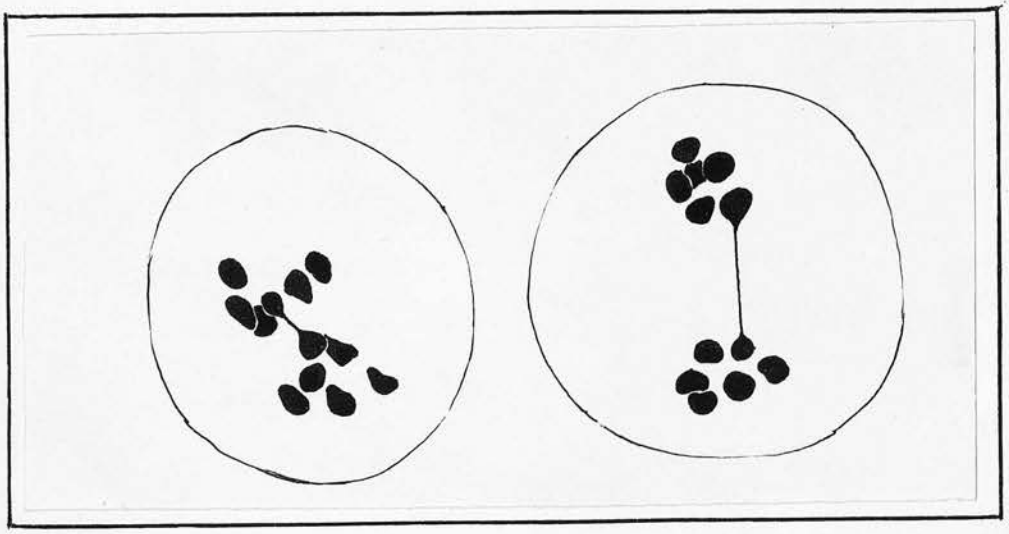


Fig. 3. Early anaphase I. delayed separation of one bivalent

Fig. 4. Anaphase I bridge.

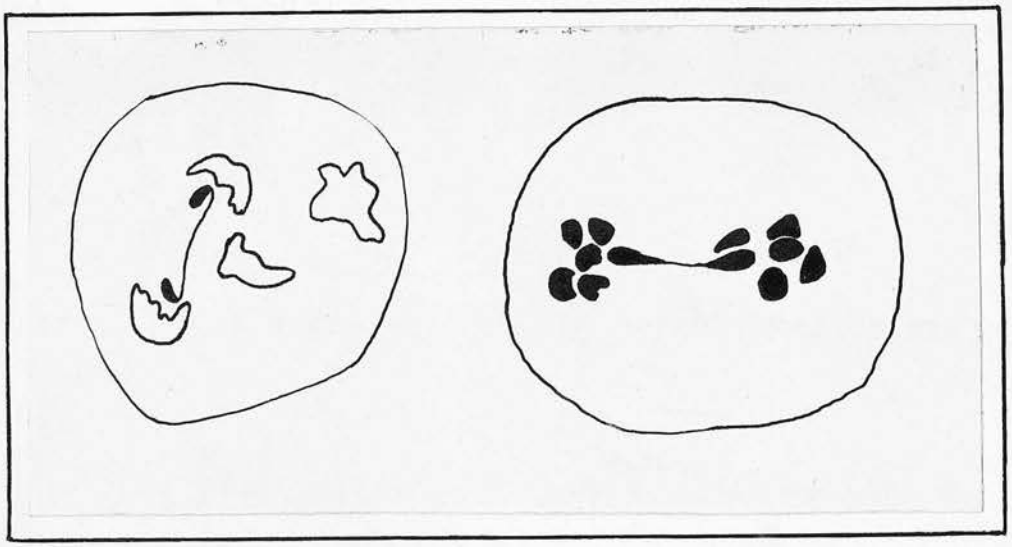


Fig. 5. Anaphase II bridge.

Fig. 6. Delayed separation of bivalent at anaphase I.

MEIOTIC STUDIES.

(1) Diploid population samples

(a) P. carinata (2n = 12). Plate II, figs. 1-6.

The material available for study had been fixed in S 2 and sectioned. Stages examined were first metaphase and anaphase.

First Metaphase. Figs. 1 and 2. Six bivalents were always present, one rather short, the remainder being approximately similar in size. The chiasmata were usually single and terminal, giving simple, rod bivalents, but occasionally one or two ring bivalents with two terminal chiasmata were met with. Usually one, and occasionally two or three, rod bivalents had the chiasma slightly sub-terminal, though quite frequently all the bivalents had a completely terminal chiasma. The exact chromosome association observed was as follows:-

No. of bivalents per cell		No. of x^{ta} per cell	No. of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$			
6	-	6	85	510
5	1	7	35	245
4	2	8	8	64
	Totals		128	819

Average chiasma frequency per bivalent = 1.07

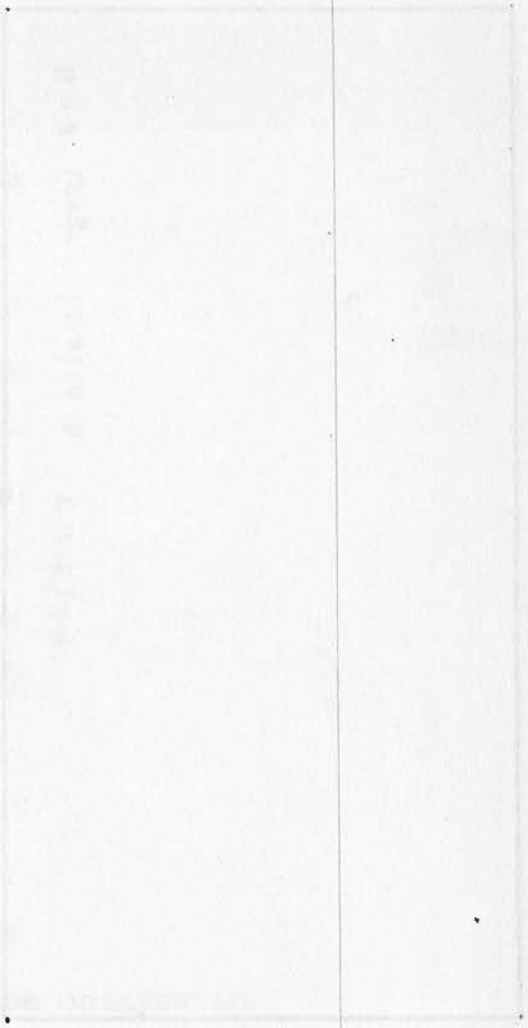


PLATE III.

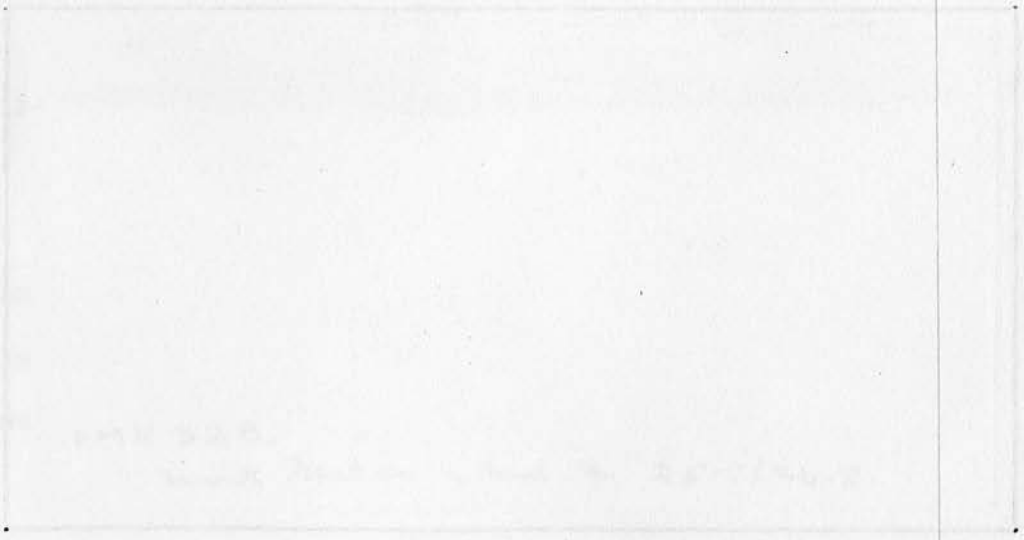
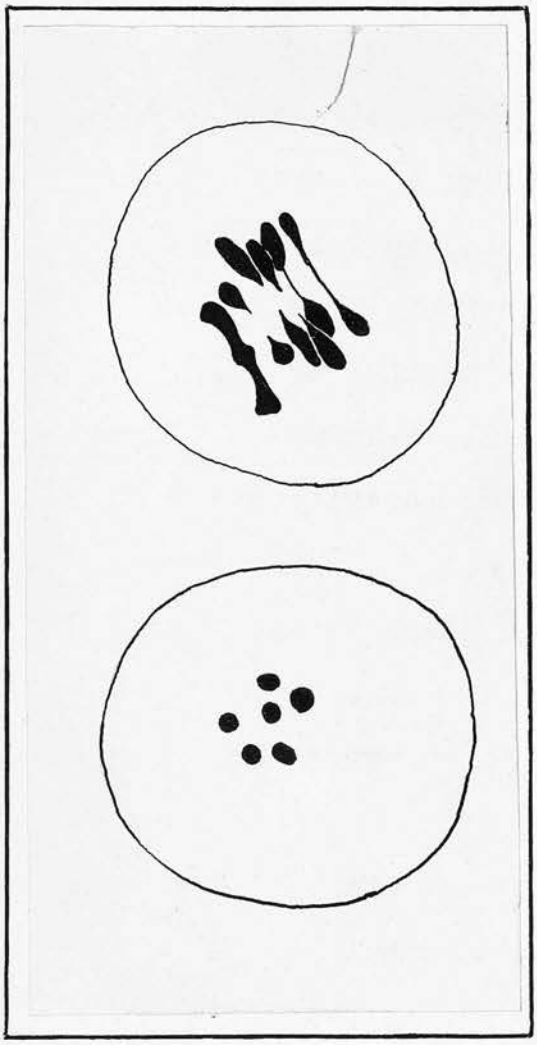
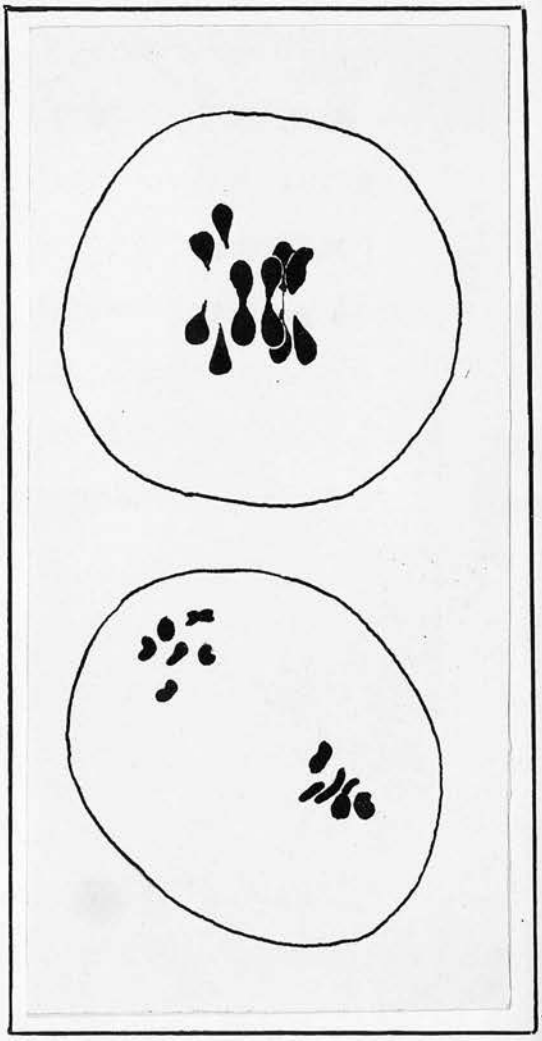


PLATE III.

PsS9





Figs. 7 and 8 (above) Early anaphase.
Lagging bivalents.

Fig. 9. Metaphase
Polar view.

Fig. 10. Metaphase II.
Fixative 2BE.

P82.

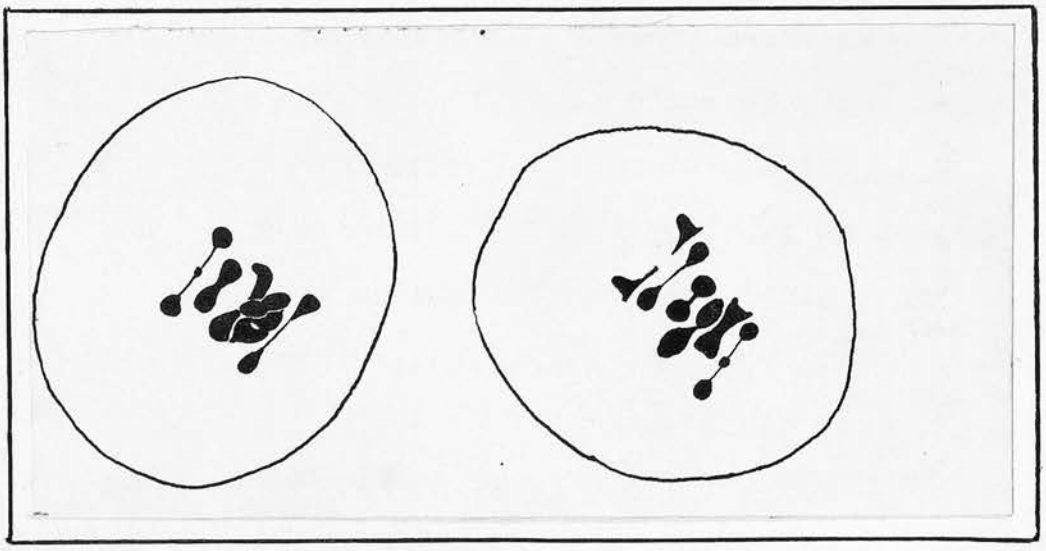


Fig. 11. Late metaphase.

Fig. 12. Early anaphase I.

Anaphase. Figs. 3-6. In about half the cells observed one or two bivalents showed a delayed separation at early anaphase. Later, in the great majority of cases the separation was entirely free and always uniform with six chromosomes passing to each pole. Among a large number of cells examined at late anaphase, five were seen in which separating chromosomes of one bivalent remained connected by a "bridge" across the equator. The acentric fragment expected in association with such bridges, if they represent the result of crossing over in an inversion, was not seen.

Second anaphase. No abnormality was observed in the majority of cells examined, but in two cells one chromosome was seen to be delayed in separation compared with the others, and in three cases a definite bridge was found.

(b) PsS9 (2n = 12). Plate III, figs. 7-10.

Smears fixed in 2 BE and sectioned material fixed in Belling's Navaschin solution were examined.

Metaphase. The twelve chromosomes were always present as six bivalents. Most of the bivalents had a single chiasma but one or two ring bivalents per cell were not infrequent. Twenty-three complete cells were analysed as follows:-

No. of Bivalents per cell		No. of x^{ta} per cell	No. of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$			
6	-	6	10	60
5	1	7	9	63
4	2	8	4	32
	Totals		23	155

Average chiasma frequency per bivalent = 1.12

The chiasmata were terminal, although in half the cells one or two rod bivalents had the chiasma slightly unterminalised.

Anaphase. At early anaphase three bivalents usually separated slightly in advance of the other three, but at later stages no irregularity was found, the chromosome distribution always being 6 : 6.

Tetrad formation. In one cross, with P. alpina (Edin.), this plant, PsS9, had behaved as a tetraploid, presumably producing diploid pollen. Investigation of the tetrads was undertaken, since no irregularity at earlier meiotic stages had been detected, in order to find whether any aberration existed in late spore formation. An examination of some hundreds of cells in the tetrad stage failed to show any departure from the normal condition.

PLATE IV.

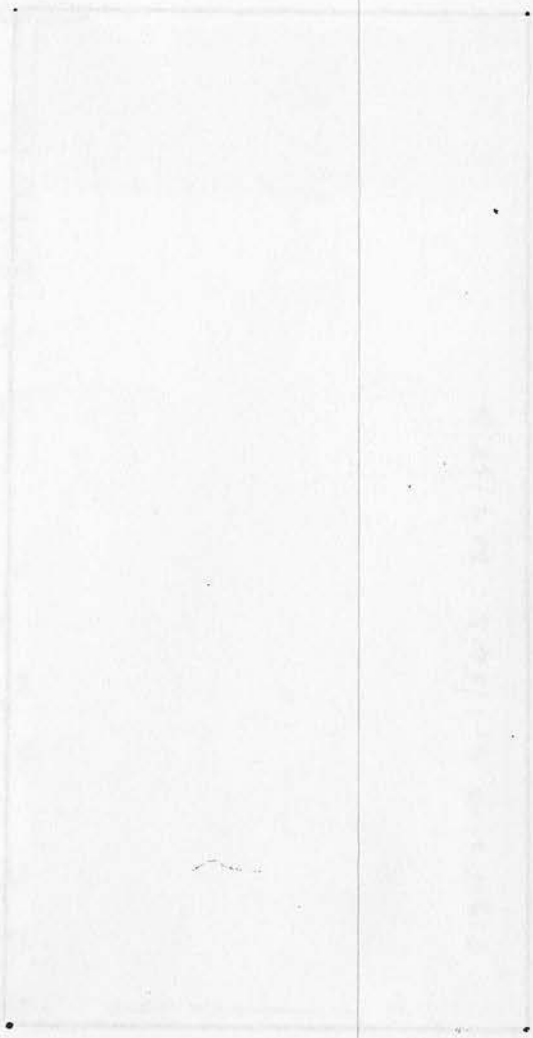


PLATE IV. P84

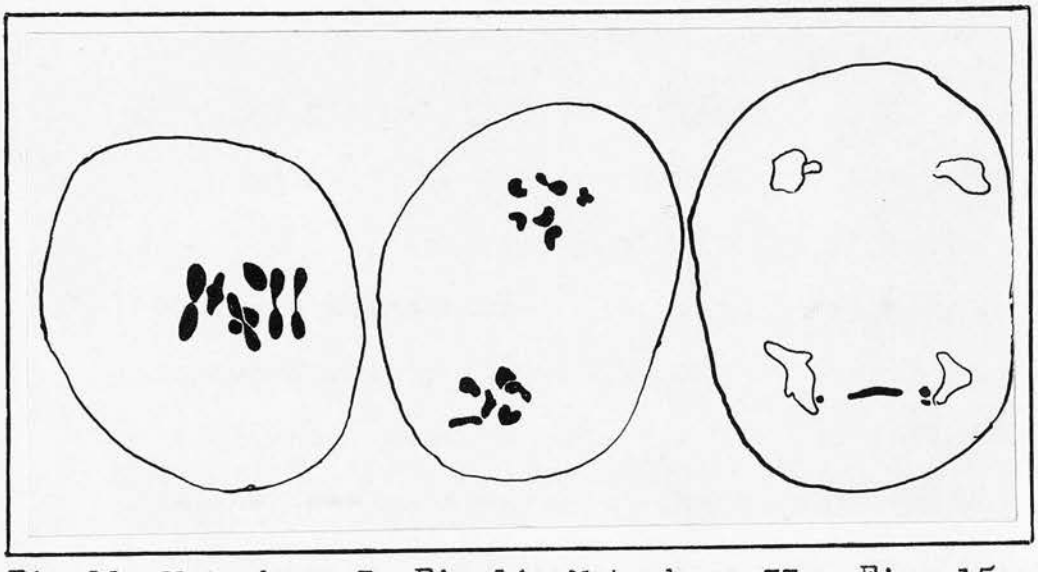


Fig.13. Metaphase I. Fig.14. Metaphase II. Fig. 15. Telophase II. Bridge.
(Smear fixed in 2BE).

P113

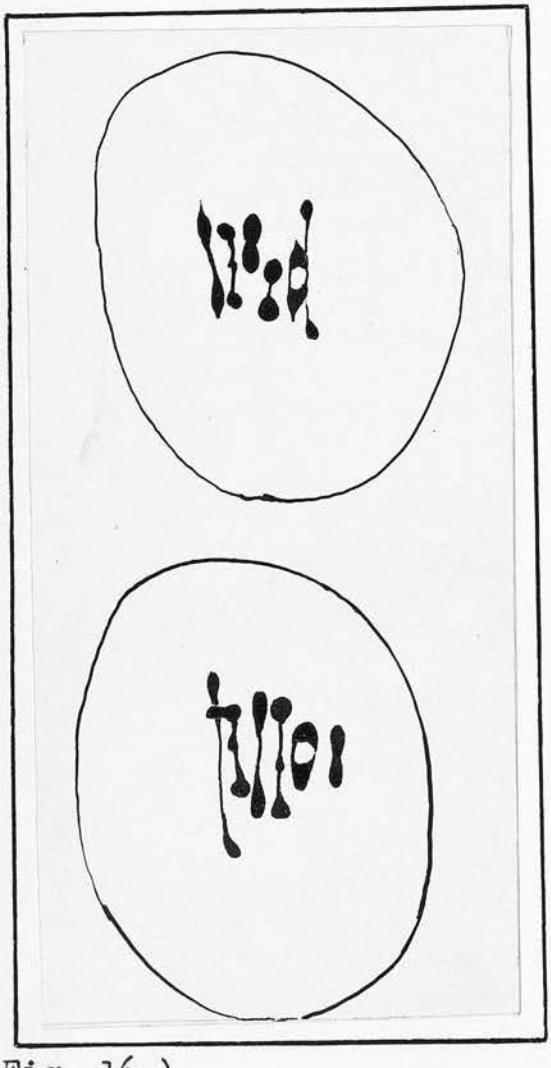


Fig. 16.) Early anaphase I.
Fig. 17.)

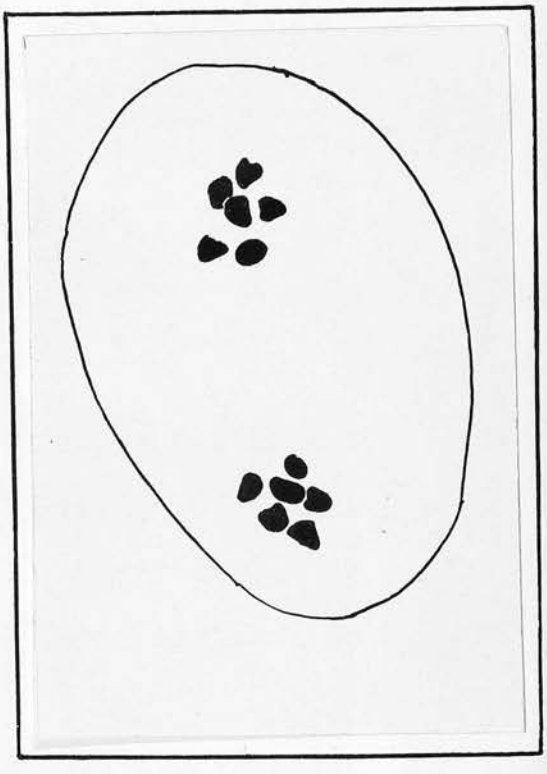


Fig. 18. Metaphase II.

(c) P82 (2n = 12). Plate III, figs. 11 and 12 (page 20)

The material examined had been fixed in Belling's Navaschin solution and sectioned.

Metaphase. Fig. 11. Twenty cells examined at this stage showed regular formation of six bivalents. Five bivalents always had a single chiasma, usually terminal, though often slightly unterminalised, while in about half the cells one ring bivalent with two chiasmata was found. The exact analysis was as follows:-

No. of bivalents per cell		No. of x^{ta} per cell	No. of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$			
6	-	6	8	48
5	1	7	12	84
	Totals		20	132

Average chiasma frequency per bivalent = 1.1

Anaphase. Fig. 12. No irregularity was observed, the bivalents always segregating regularly to give six chromosomes at each pole.

(d) P84 (2n = 12). Plate IV, figs. 13-15.

Only a very small amount of material was available as smears fixed in 2 BE. Further material could not be obtained because of the death of the plant.

Metaphase. Figs. 13 and 14. The few cells seen at this stage did not show any deviation in behaviour from the other diploid forms. Six bivalents

were formed with usually a single chiasma each. One of the six bivalents often had the chiasma unterminalised.

Anaphase. Fig. 15. No irregularity was observed at first anaphase. At second anaphase, however, in one cell a chromosome was present as a bridge across one of the spindles.

(e) P113 (2n = 12). Plate IV, figs. 16-18 (page 23).

The material was fixed in Belling's Navaschin solution and sectioned.

Metaphase. Six bivalents were always formed, and here, as elsewhere, single chiasma types predominated. Frequently one, and occasionally two bivalents with two chiasmata per cell were found.

Anaphase. No irregularity was found at either first or second anaphase.

Tetrad Formation. Examination of this stage was undertaken for the same reason as in PsS9. A diploid pollen grain of P113 appears to have fertilised an ovule of P65 to produce the tetraploid hybrid C46. No irregularity of tetrad formation was found.

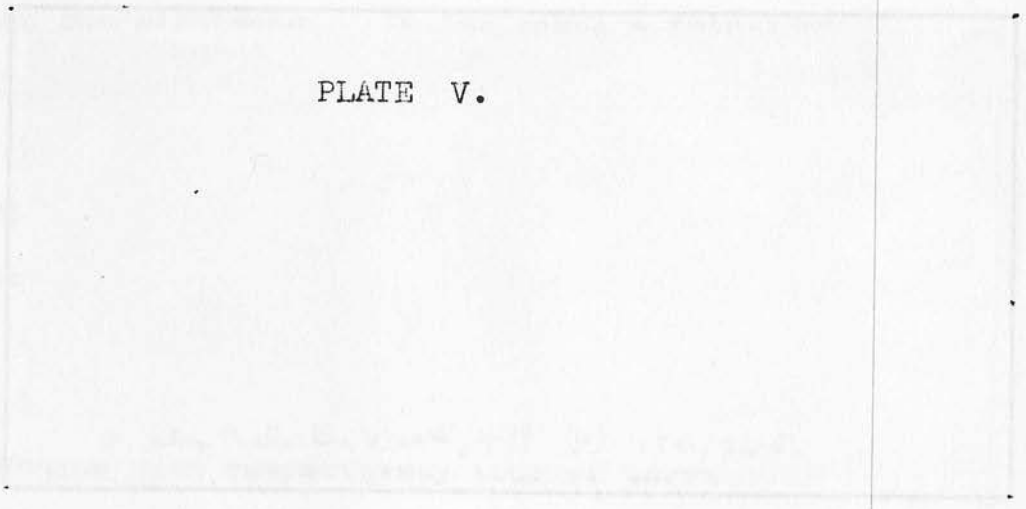


PLATE V.

PLATE V. P. ALPINA (EDIN.)

Fig. 19. Metaphase I. Chain quadrivalent.

Fig. 20. Metaphase I.

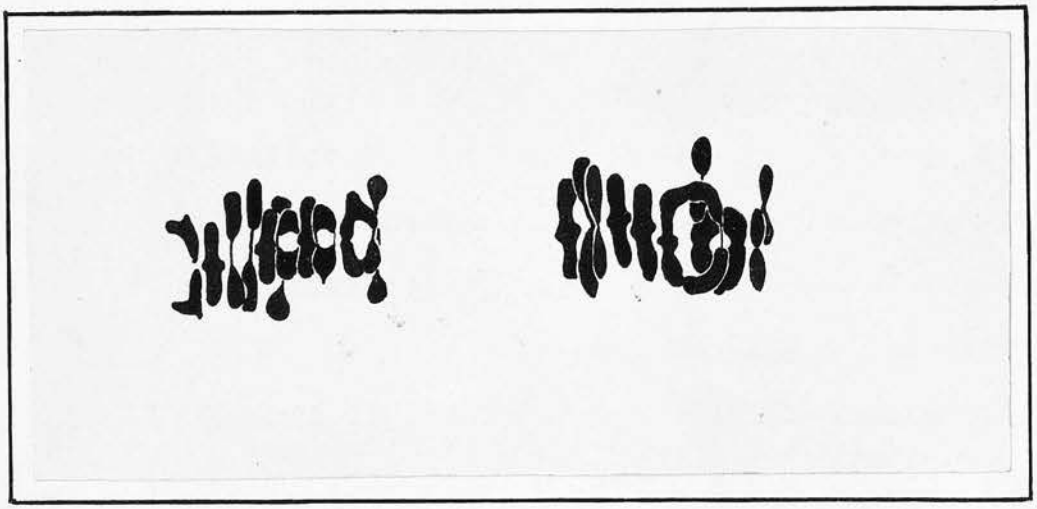


Fig. 21. Metaphase I.

Fig. 22. Metaphase I. Two ring quadrivalents.

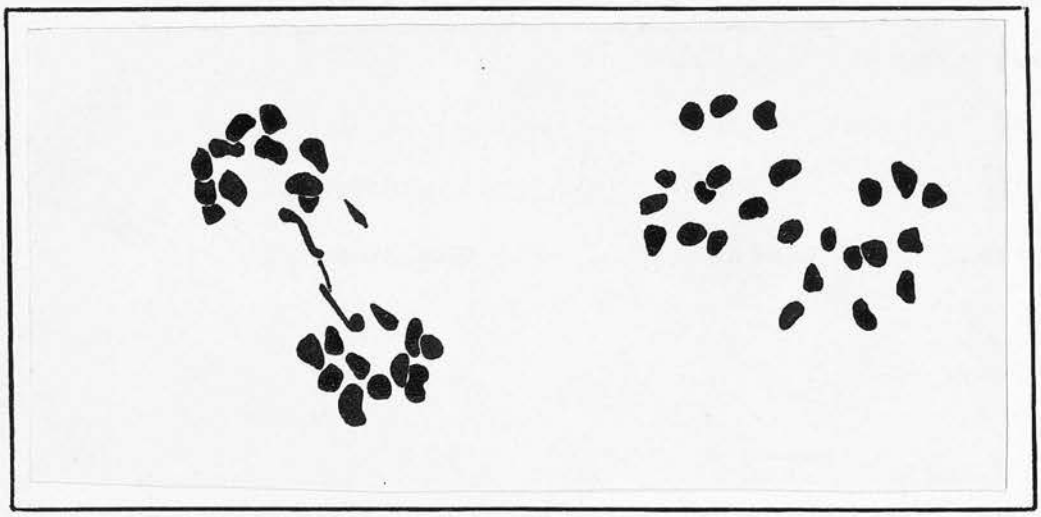


Fig. 23. Anaphase I. bridge and possible fragment.

Fig. 24. Metaphase II. (Acetocarmine smear)

(2) Tetraploid population samples.

(a) P. alpina (Edin.) (2n = 24). Plate V, figs 19-24.

Material was examined in aceto-carmine smears and in sections after fixation in Belling's Navaschin solution.

Metaphase. Figs 19-22. The chormosome associations at this stage were either twelve bivalents per cell, or ten and eight bivalents with respectively one and two quadrivalents. In two cases a trivalent and univalent was seen.

The bivalents usually had a single chiasma, most often terminal but frequently sub-terminal. One or two ring bivalents per cell with two terminal chiasmata occurred fairly often.

The quadrivalents found were either simple rings or chains with respectively four or three terminal chiasmata. So far as could be determined, unterminalised chiasmata were very rare in multivalents. One case is seen in fig. 22.

Fifty-four cells were examined in detail at metaphase or early anaphase in the aceto-carmine preparations. The associations were analysed as follows:-

Bivalents		Quadrivalents chains	rings	Trivalents	x^{ta} per cell	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$	$3x^{ta}$	$4x^{ta}$	$2x^{ta}$			
12	-	-	-	-	12	11	132
11	1	-	-	-	13	11)	
10	-	1	-	-	13	5)	234
9	1	-	-	1	13	2)	
10	2	-	-	-	14	6)	
9	1	1	-	-	14	3)	266
10	-	-	1	-	14	10)	
9	1	-	1	-	15	5	75
8	-	-	2	-	16	1	16
			Totals			54	723

Average chiasma frequency per potential bivalent = 1.11

Inspection of nuclei at diakinesis confirmed the conclusions regarding chromosome association, detailed above at metaphase. No more than two multivalents per cell were observed and this comparatively rarely. The nuclei at this stage were unfortunately too crowded for accurate drawing.

Anaphase. Figs. 23-24. Quite frequently one or two bivalents showed a delayed separation at early anaphase. Later the very great majority of cells had a clean segregation but in two cells a single chromatid bridge was found with a probable fragment. In one further cell two broken bridges were seen but no fragments appeared to be present.

The numerical segregation was found to be predominantly regular; nine cells showed a 12:12 distribution and one 11:13.

PLATE VI.

PLATE VI. P63

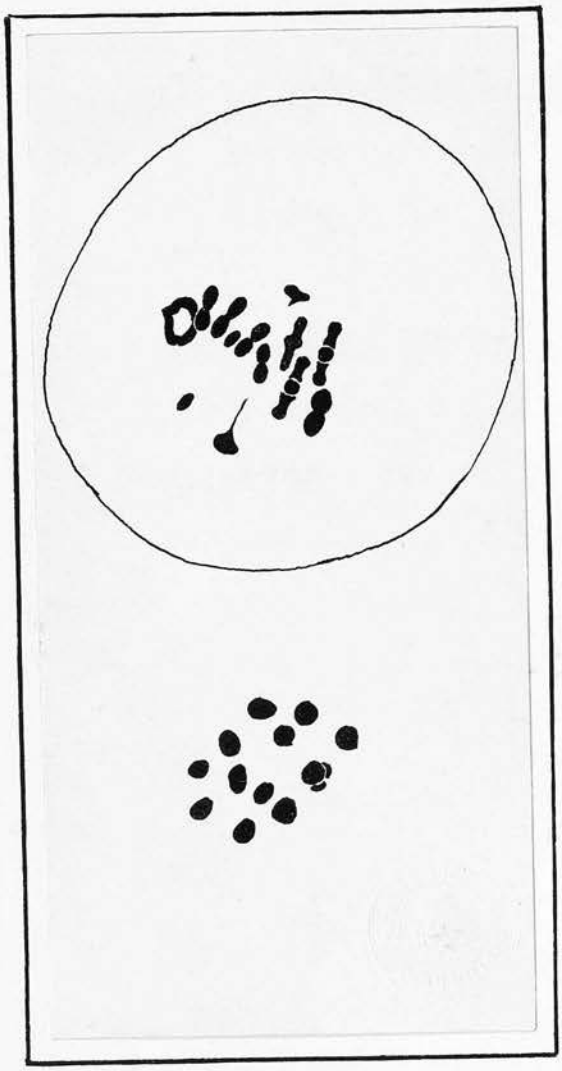


Fig. 25. (Left) Metaphase I.
Precocious separation
of two bivalents.
One ring quadrivalent.

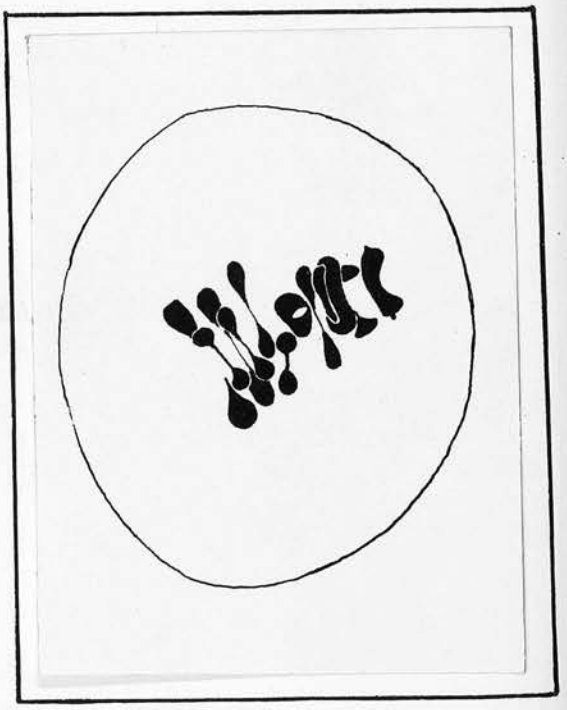


Fig. 26. Metaphase I.
Polar view.

Fig. 27. Metaphase I.
one ring quadrivalent.

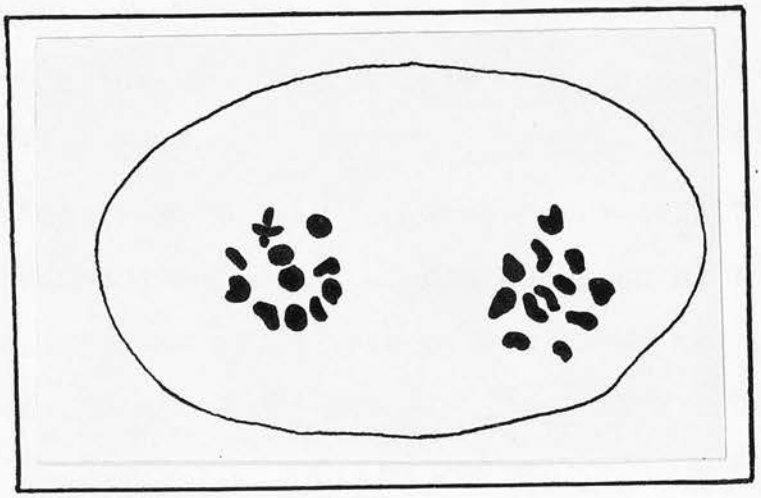


Fig. 28. Metaphase II.
Twelve chromosomes
at each pole.

NOTE. All the above fixed in S_2

(b) P63 (2n = 24). Plate VI, figs. 25-28.

The available preparations were sections following fixation of the material in Belling's Navaschin solution.

Metaphase. Figs. 25-27. The chromosomes were associated as bivalents or with one, occasionally two, quadrivalents. Only in one case was a trivalent and univalent found. Fourteen complete complements were analysed in side view. The different associations occurred with frequencies as follows:-

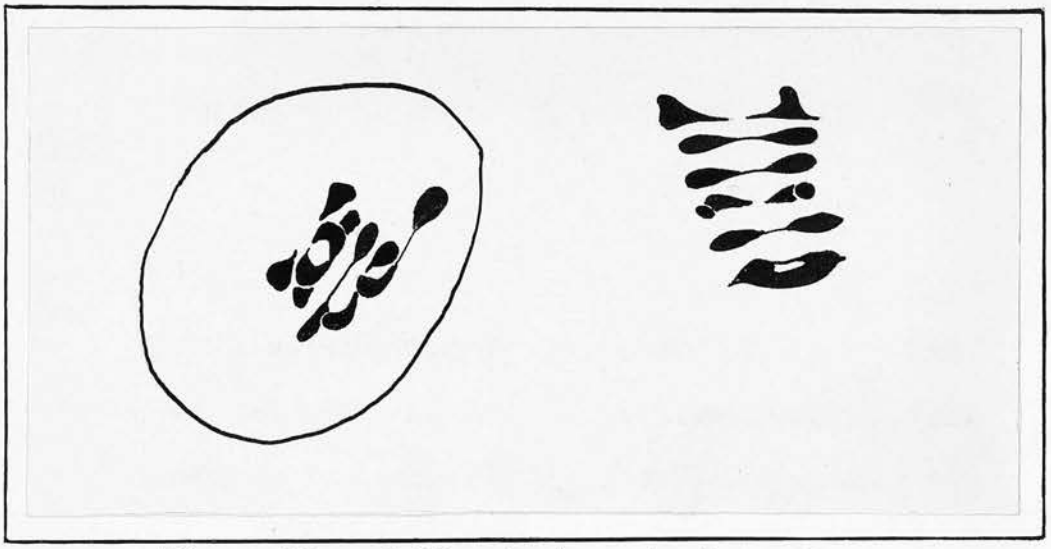
Bivalents		Quadrivalents chains	rings	Trivalents	x^{ta} per cell	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$	$3x^{ta}$	$4x^{ta}$	$2x^{ta}$			
12	-	-	-	-	12	7	84
11	1	-	-	-	13	12)	
10	-	1	-	-	13	2)	182
10	-	-	1	-	14	3)	
8	-	-	1	1	14	1)	56
9	1	-	1	-	15	1	15
			Totals			26	337

Average chiasma frequency per potential bivalent = 1.08

Anaphase. Fig. 28. The numerical distribution was accurately determined in 12 cells. All had twelve chromosomes at each pole. In these cells and in numerous others where the chromosome distribution could not be counted no anaphase bridges were found. In fact, the only irregularity noticed at anaphase was a tendency for a few bivalents - two

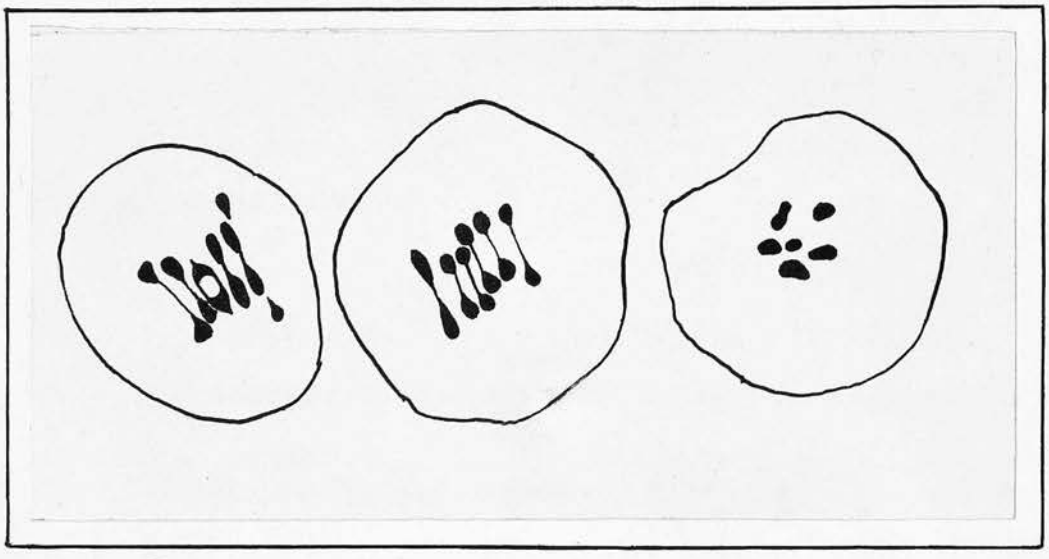
PLATE VII.

PLATE VII. C28,(P. CARINATA x PsS9).



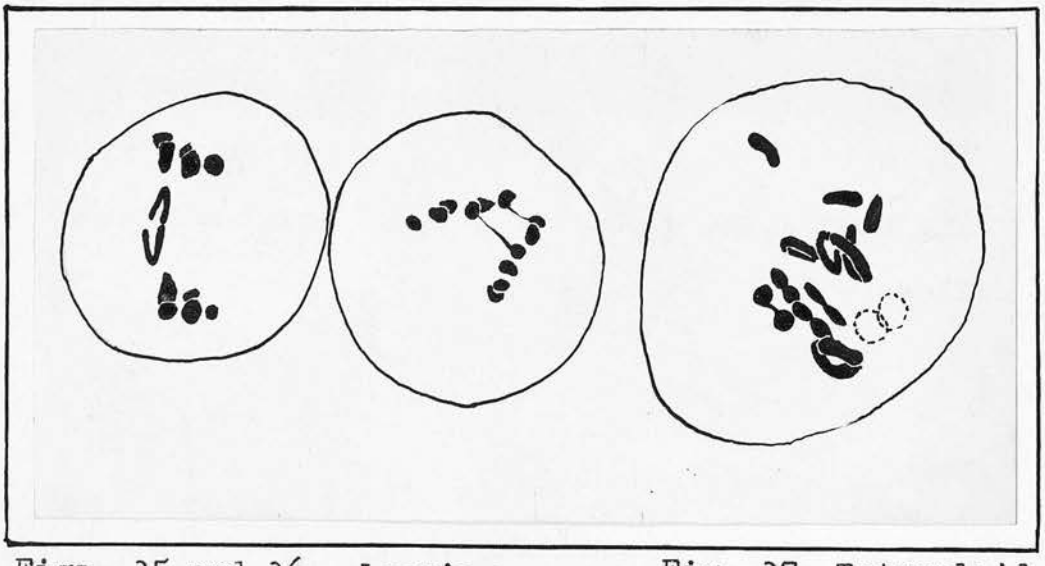
Figs. 30 and 31. Late metaphase I.

C29 (PsS9 x P84).



Figs. 32 and 33. Late metaphase I.

Fig. 34. Metaphase I. Polar view.



Figs. 35 and 36. Lagging bivalents at Anaphase I.

Fig. 37. Tetraploid P.M.C.

or three - to segregate before the remaining chromosomes.

(c) P65 (2n = 24).

Only a small amount of rather poorly fixed material was available for examination. So far as could be seen the behaviour at metaphase and anaphase was similar to that observed in P63.

(3) Diploid hybrids.

(a) C28.(P.carinata x PsS9). Plate VII, figs 30-31.

The material had been fixed in Belling's Navaschin solution and sectioned.

Diakinesis and Metaphase. Figs. 30-31.

In the cells examined at diakinesis and metaphase the chromosomes were always present as six bivalents. Most usually there were in each cell five rod bivalents with terminal chiasmata and a ring bivalent with two chiasmata. The ring bivalents never, and the rod bivalents only very rarely, had sub-terminal chiasmata. Occasionally cells with six rod bivalents, or four rod and two ring bivalents, were found. The exact analysis was as follows:-

No. of Bivalents per cell		No. of x^{ta} per cell	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$			
6	-	6	50	300
5	1	7	62	434
4	2	8	13	104
	Totals		125	838

Average chiasma frequency per bivalent = 1.12

Anaphase. No irregularity was observed. The separation was quite clean and always numerically equal.

(b) C29 (2n = 12),(PsS9 x P84). Plate VII, figs 32-37 (page 31)

Material examined had been fixed in 2 BE and sectioned.

Metaphase. Figs. 32-34. The chromosomes were always present as six bivalents. The bivalents normally had a single terminal or nearly terminal chiasma, but one or two ring bivalents were found in some cells. The analysis of 110 complete cells was as follows:-

No. of Bivalents per cell		No. of x^{ta}	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$			
6	-	6	49	294
5	1	7	55	385
4	2	8	6	48
	Totals		110	727

Average Chiasma frequency per bivalent = 1.10

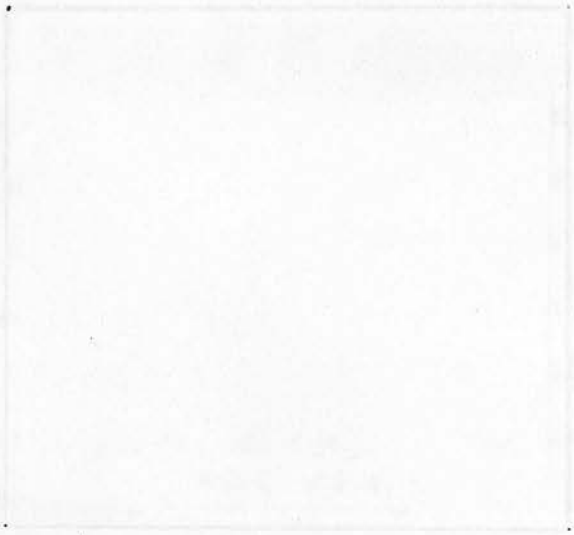


PLATE VIII.

PLATE VIII. C21,(P. ALPINA (EDIN.) X PsS9).

Fig. 38. Late metaphase I.
One ring quadrivalent.

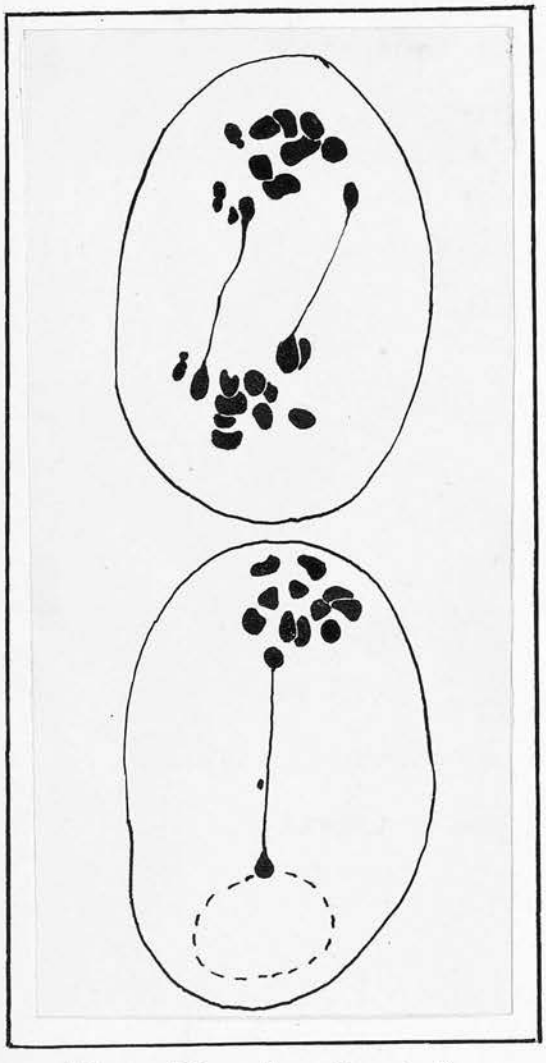


Fig. 40. Anaphase I.
Single bridge
with fragment.

Fig. 39. (Left). Anaphase I.
Two separate bivalents
forming bridges.

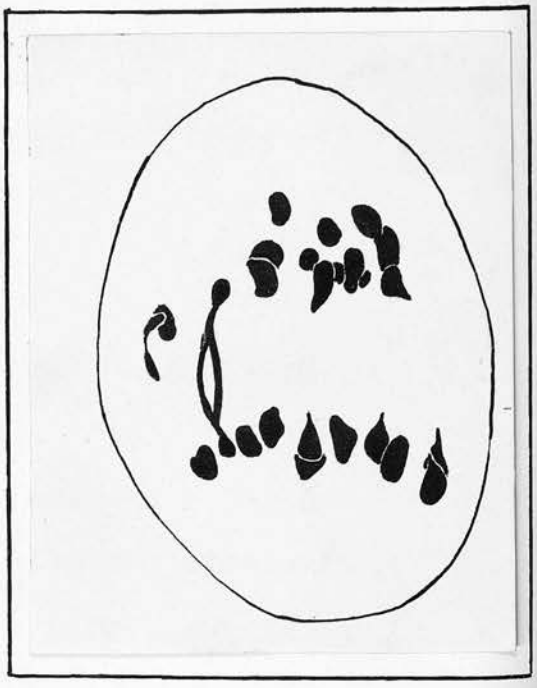


Fig. 41. Anaphase I.
Double bridge from one
bivalent with two (?)
fragments.

All from aceto-carmine smears.

In one cell at a pre-metaphase stage twenty-four chromosomes could be counted with a considerable degree of certainty (Fig. 37). In addition there were three non-chromosomal bodies, not stained so intensely as the chromosomes. They were probably of nucleolar nature. It seems certain that this was a tetraploid pollen mother cell resulting from doubling of the chromosome number in an archesporial cell.

Anaphase. Figs. 35 and 36. At early anaphase lagging of one or two bivalents was seen. Later, however, the bivalent chromosomes separated quite cleanly and there were no irregularities, numerical or otherwise.

(4) Tetraploid hybrids.

(a) C21 (2n = 24), (P. alpina (Edin.) x PsS9).
Plate VIII, figs 38-41.

The material was examined in aceto-carmine smears.

Metaphase. Fig. 38. The behaviour was very similar to that of other tetraploid forms, the chromosomes being present as bivalents or with at most 1-2 quadrivalents per cell. In one case a trivalent and univalent was seen.

The majority of bivalents had a single chiasma but as many as two ring bivalents occurred in some cells. The quadrivalents were usually simple rings, a chain of four being found only in two cells. Twenty-three complete cells were analysed as follows:-

Bivalents	Quadrivalents	Trivalents		x^{ta} per cell	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$	$3x^{ta}$	$4x^{ta}$	$2x^{ta}$			
12	-	-	-	-	12	5	60
11	1	-	-	-	13	7	91
10	-	1	-	-	13	3	39
10	2	-	-	-	14	1	14
10	1	1	-	-	14	1	14
10	-	-	1	-	14	3	42
9	1	-	1	-	15	1	15
8	2	-	1	-	16	1	16
8	-	1	-	1	14	1	14
			Totals			23	305

Average chiasma frequency per potential bivalent = 1.105

Anaphase. Figs 39-41. The numerical distribution at anaphase was predominantly regular. The maximum unbalance found was an 11:13 distribution, and occasionally one univalent was greatly delayed in segregation. Twenty cells had the following distribution:-

15 cells 12:12
2 cells 13:11
3 cells 12:11 + 1 lagging chromosome.

In addition to these cells some two hundred other cells were examined in order to determine the incidence of other irregularities. In three cells a single bridge was found, accompanied in two cells by

PLATE IX.

PLATE IX. C22, (P. ALPINA (EDIN.) x P63)

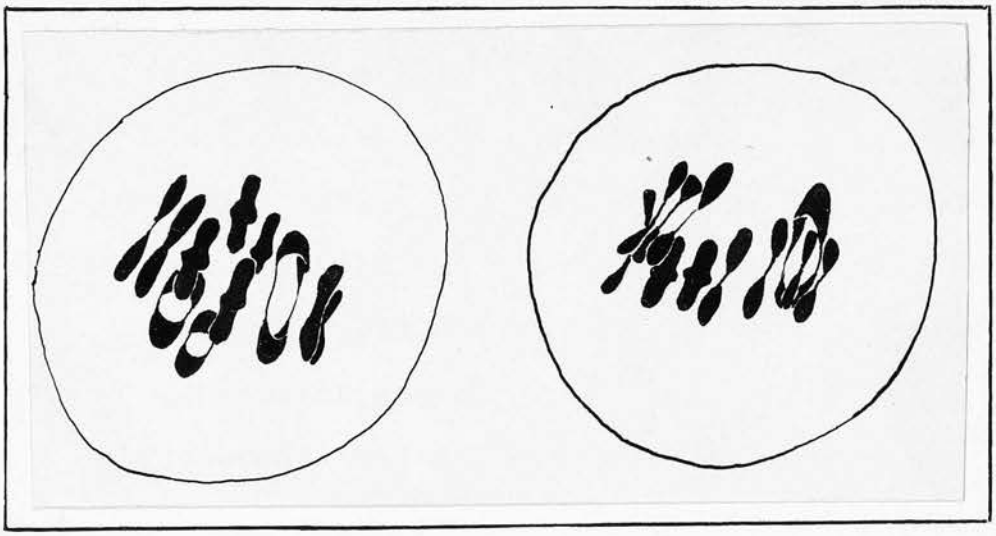


Fig. 42. Late metaphase I. One ring quadrivalent and two ring bivalents.

Fig. 43. Late metaphase I.

Fig. 44. Late metaphase I.

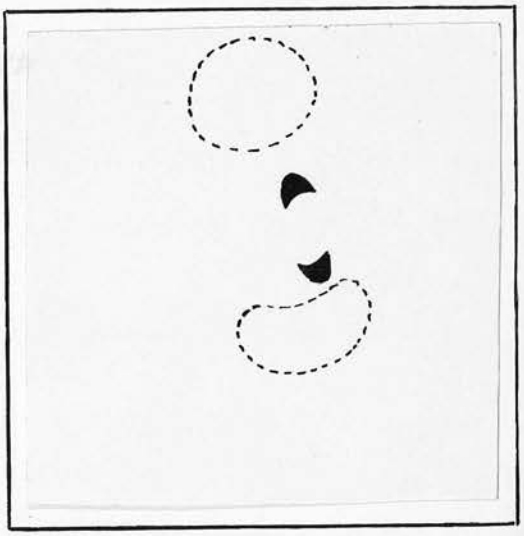


Fig. 45. Telophase I. Lagging bivalent.

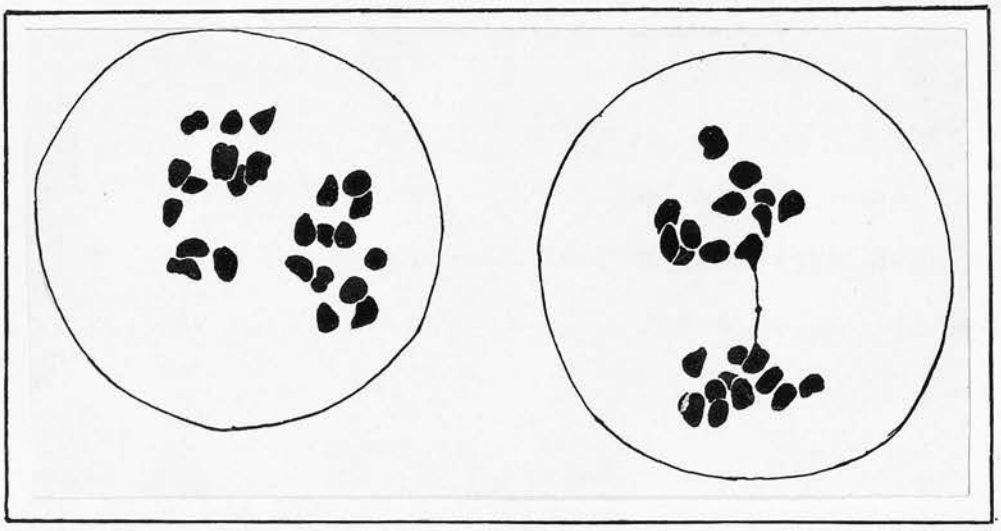


Fig. 46. Metaphase II.

Fig. 47. Anaphase I. Bridge.

a chromatid fragment (Fig. 40). In one other cell two bridges involving separate bivalents were found but fragments could not be seen (Fig. 39). Also one case of a double bridge involving one bivalent was found (Fig. 41). Here a chromosome fragment was lying adjacent to the bridge close to a univalent. It was not possible to determine whether the junction between the two halves of the fragment body was an actual connection, or whether, in fact, the body was one fragment or two as expected with a double inversion bridge.

At second anaphase a cell was found showing a single chromatid bridge.

(b) C22 (2n = 24), (P. alpina (Edin.) x P63). Plate IX, figs 42-47.

The material was fixed in Belling's Navaschin solution and sectioned.

Metaphase. Figs. 42-44. The chromosomes were present as twelve bivalents in one cell observed, elsewhere one or two quadrivalents appeared. Trivalents were not found in the cells examined. The bivalents occasionally had two chiasmata and the quadrivalents were either chains or rings of four. The associations were analysed in twelve cells as follows:-

Bivalents		Quadrivalents		x^{ta} per cell	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$	$3x^{ta}$	$4x^{ta}$			
12	-	-	-	12	1	12
11	1	-	-	13	4)	
10	-	1	-	13	2)	78
10	-	-	1	14	4	56
8	2	-	1	16	1	16
			Totals		12	162

Average chiasmata per potential bivalent = 1.12

The chiasmata were frequently slightly sub-terminal in the one-chiasma bivalents, but very rarely so in ring bivalents or in quadrivalents.

The types of association described above were confirmed by observation of nuclei at diakinesis.

Anaphase. Figs. 45-47. Segregation was effected cleanly in most cases examined (93/106), but in eleven cells the chromosomes of one bivalent remained connected by a bridge. A fragment was not seen to accompany the bridge in any of these cases. In one cell a ring bivalent showed very delayed separation, though this was without bridge formation.

The numerical distribution was regular giving polar nuclei with twelve chromosomes in 23/24 cells examined and in the remaining cell it was 11:13.

PLATE X.

PLATE X. C37, (P. ALPINA (EDIN.) x P81)

Figs. 48-51
Metaphase I.

Fig. 48. Four trivalents.

Fig. 49. Five trivalents.

Fig. 50. Four trivalents.

Fig. 51. Six bivalents.

All aceto-carmine preparations.

(5) Triploid hybrids.

(a) C37 (2n = 18), (P. alpina (Edin.) x P81). Plate X, figs 48-51.

The material available was smears fixed in 2 BE and in aceto-carmine.

Metaphase. Figs. 48-51. The associations found were trivalents or bivalents with univalents. A maximum number of five trivalents was found in several cells and in one cell only bivalents with six univalents were present. Only nine cells could be completely analysed.

Univalents	Bivalents	Trivalents		x^{ta} per cell	No. of cells	Total x^{ta}
$0x^a$	$1x^a$	$2x^{ta}$	$3x^{ta}$			
1	-	1	3	2	14	2	28
1	-	1	5	-	12	3	36
2	2	-	3	1	11	1	11
2	2	-	4	-	10	1	10
4	3	1	2	-	9	1	9
6	4	2	-	-	8	1	8
			Totals			9	102

Average No. of $\frac{1}{2}$ x^{ta} per chromosome = 1.26

The trivalents were either simple chains of three chromosomes or a ring of two with one attached. In the trivalents of the latter type illustrated (Figs. 48 and 49) it was difficult to decide whether the third chromosome was attached at a triple chiasma or whether it was attached interstitially to one in a translocated region as was clearly shown in the

PLATE XI.

PLATE XI. C38, (P. ALPINA (EDIN.) x P84)

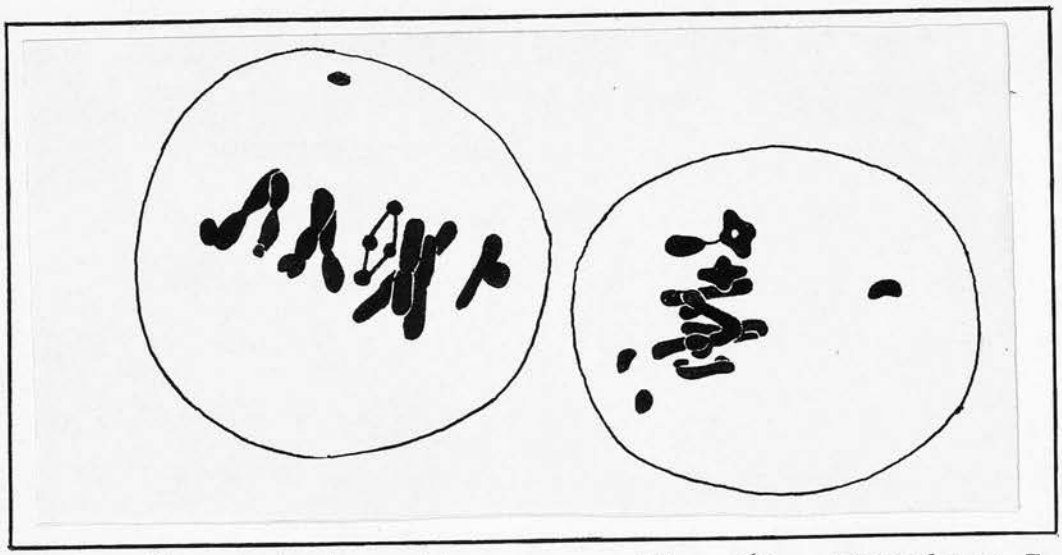


Fig. 52. Metaphase I.
Five trivalents.

Fig. 53. Metaphase I.
Top trivalent showing
effect of translocation.

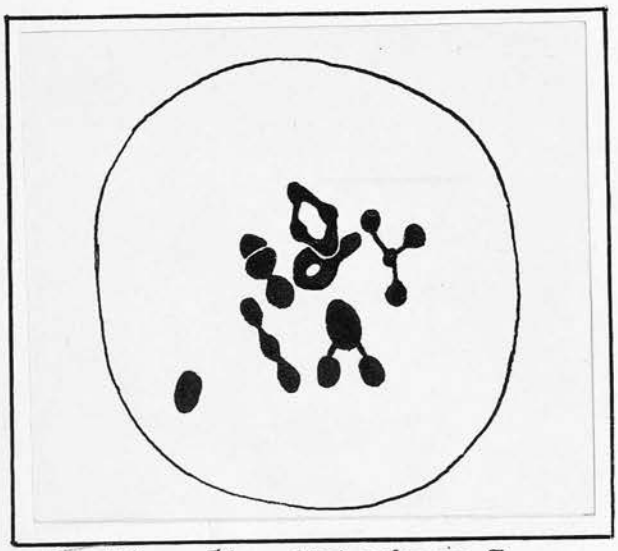


Fig. 54. Metaphase I.
Five trivalents.

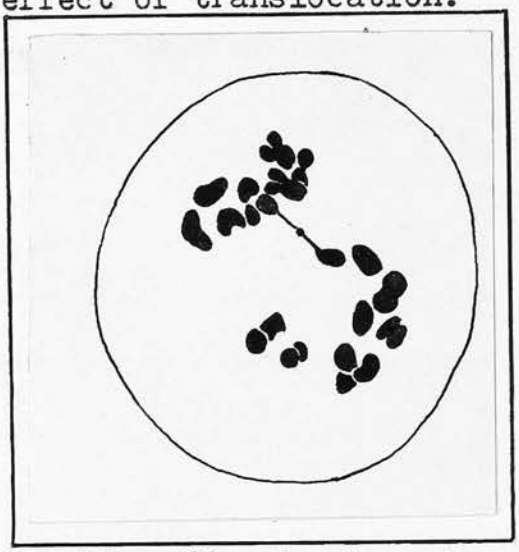


Fig. 55. Anaphase I.
bridge.

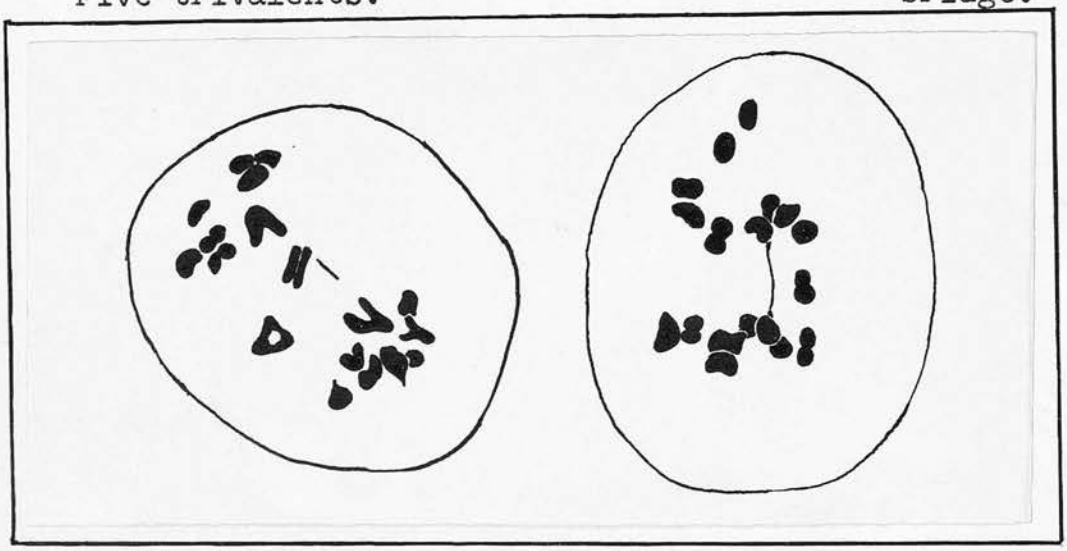


Fig. 56. Anaphase I.
Lagging bivalents
and (?) broken bridge.

Fig. 57. Anaphase I.
bridge.

(53, 56 and 57 Belling - rest aceto-carmine)

related triploid C38.

Anaphase. At this stage lagging chromosomes were frequently seen. Exact counts for chromosome distribution were obtained in only nine cells. They were as follows:-

9 - 9		in 3 cells
8 - 9	with one lagging	in 3 cells
8 -10		in 1 cell
8 - 8	with two lagging	in 1 cell
6 - 9	with three lagging	in 1 cell.

The univalents appear to segregate at anaphase I without dividing.

(b) C38 (2n = 18),(P. alpina (Edin.) x P84). Plate XI, figs 52-57.

The material examined was chiefly aceto-carmine smears with some sections made after fixation in Belling's Navaschin solution.

Metaphase. Figs. 52-54. The chromosomes were associated in trivalents or bivalents with univalents. The maximum association observed was six trivalents, seen in three cells, and the minimum was one trivalent with five bivalents with univalents, seen in one cell.

Univalents	Bivalents	Trivalents		x^{ta} per cell	No.of cells	Total x^{ta}
$0x^a$	$1x^a$	$2x^{ta}$	$2x^{ta}$ $3x^{ta}$			
-	-	-	5 1	13	3	39
1	-	1	5 -	12	1	12
1	-	1	4 1	13	2	26
1	1	-	4 1	12	1	12
2	2	-	4 -	10	1	10
2	1	1	3 1	12	1	12
3	2	1	2 1	11	2	22
3	2	1	3 -	10	2	20
4	2	2	1 1	11	1	11
5	4	1	1 -	8	1	8
			Totals		15	172

Average number of $\frac{1}{2}$ x^{ta} per chromosome = 1.27

The most frequent trivalent type was a chain requiring two chiasmata, but three chiasmata types were found, the chromosomes forming a ring of two with the third associating in a triple chiasma at the side. In two cells, however, one chromosome was attached by a terminal chiasma interstitially with one chromosome, of a ring pair joined by terminal chiasmata (Fig. 53). This type of association must result from a translocation of a terminal segment to an interstitial position. A chiasma in the critical region between chromosomes heterozygous for the translocation would be unable to terminalise in one chromosome and thus produce the observed configuration.

Apart from the trivalent just described chiasmata in tri- and bivalents were usually terminalised. Slightly unterminalised chiasmata were,

PLATE XII.

C39, (P. ALPINA (EDIN.) x P91).

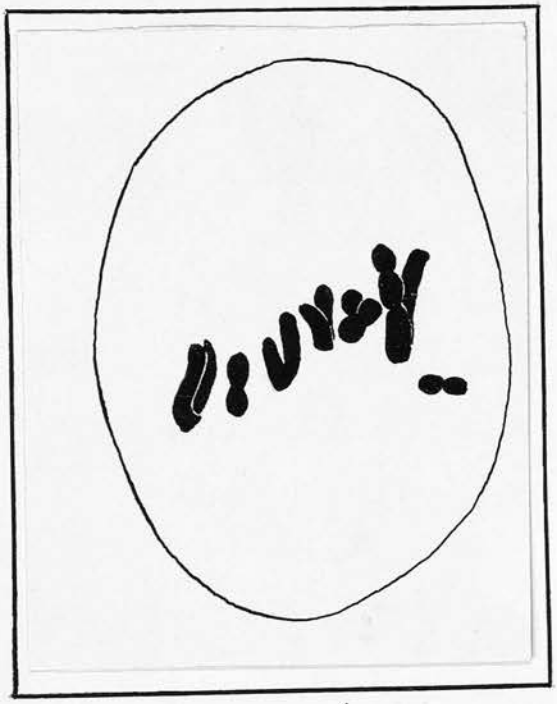


Fig. 58. Metaphase I.
five trivalents.

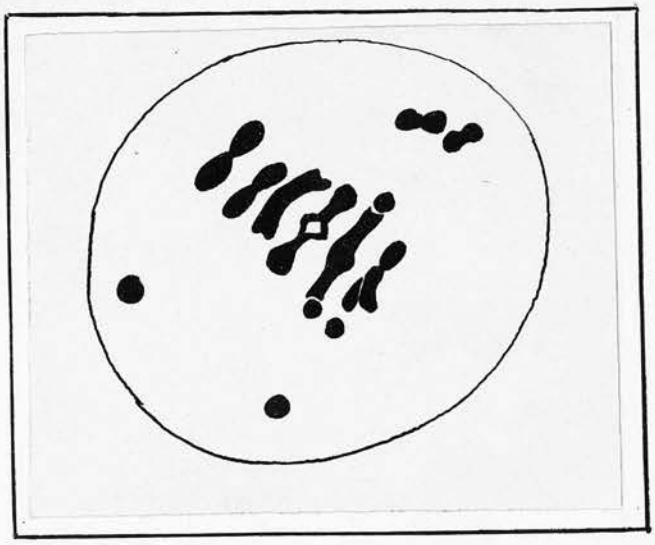


Fig. 59. Metaphase I.
One trivalent.

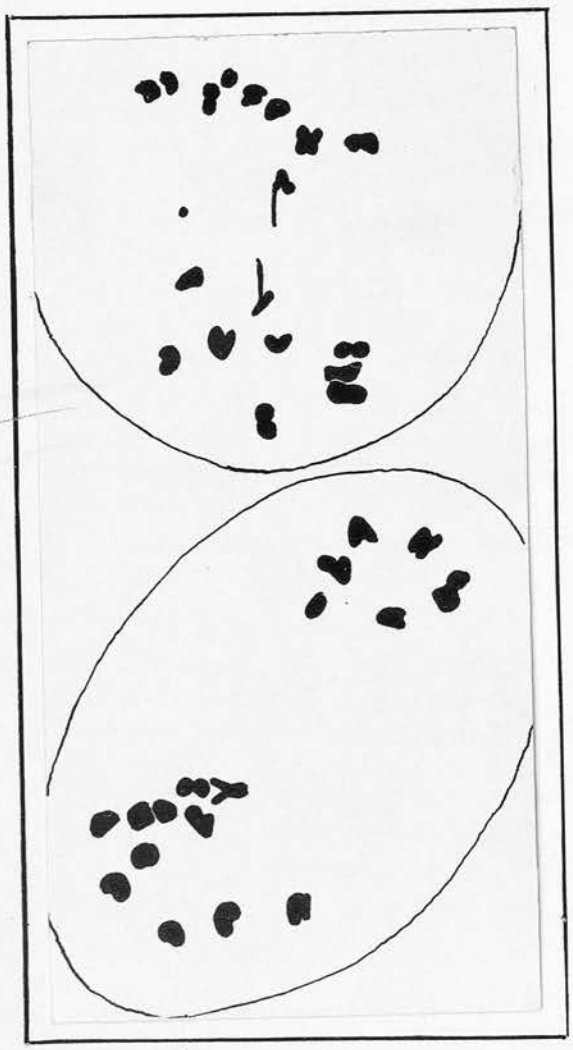


Fig. 61. Metaphase II.
12:6 chromosomes.

Fig. 60. Anaphase I.
Broken bridge
and fragment.

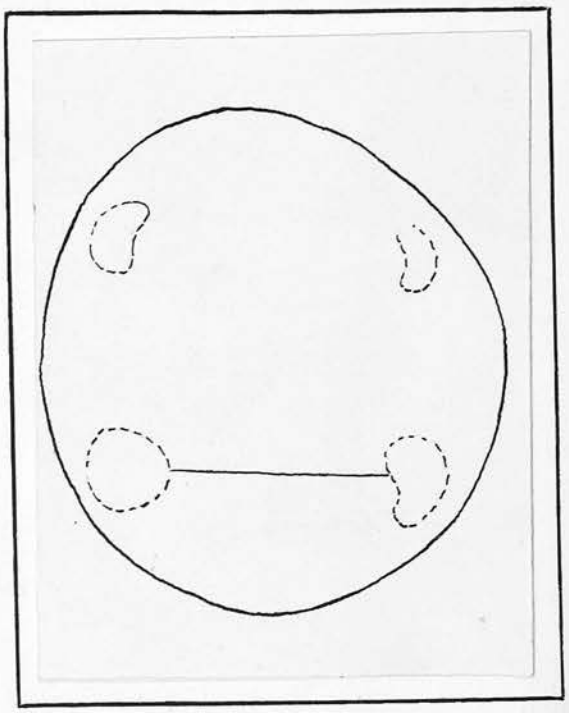


Fig. 62. Telophase II.
bridge.

however, not uncommon.

The chromosome distribution could be counted only in a few cells. The chromosomes showed equal 9:9 segregation or 9:8 and 8:8 with respectively 1 and 2 lagging univalents.

Bridges without fragments were found in three cells and a probable broken bridge in another (Fig. 56).

(c) C39 (2n = 18), (P. alpina (Edin.) x P91).
Plate XII, figs. 58-62.

The material was studied only from aceto-carmine smears.

Metaphase. (Figs. 58 and 59). Only a few cells were observed. The configurations did not differ from those observed in the related types C37 and C38. The trivalents with three chiasmata were, however, all normal with terminalised chiasmata.

First anaphase. (Figs. 60-62). Univalents segregated later than the paired chromosomes, appearing always to pass to the poles without dividing. The numerical distribution was counted in twelve cells and varied as follows:-

9 - 9		in 2 cells
9 - 8	with one lagging	in 2 cells
9 - 7	with two lagging	in 1 cell
9 - 8	with two lagging	in 1 cell
10 - 8		in 4 cells
10 - 6	with two lagging	in 1 cell
12 - 6		in 1 cell

PLATE XIII.

C38 N.S. (C38 OPEN POLLINATED).

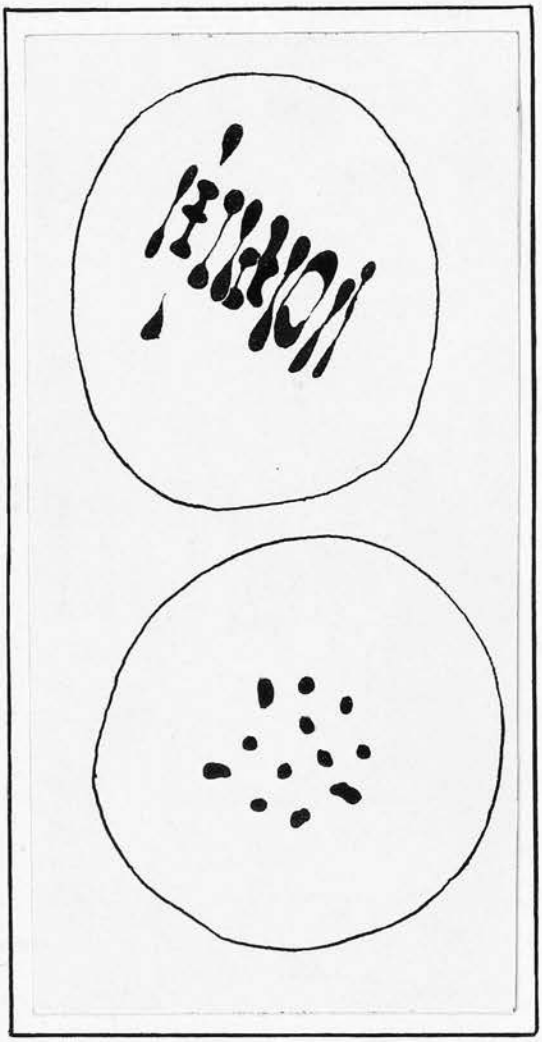


Fig. 63. Late metaphase I.

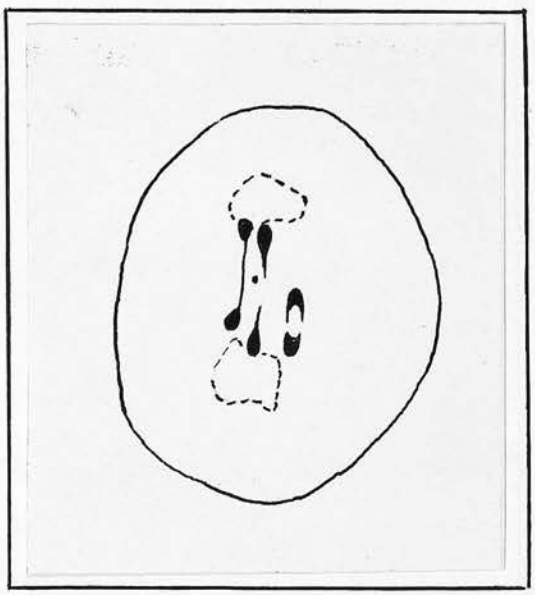


Fig. 64. Metaphase I. Polar view.

Fig. 65. Telophase I. Bridge and two lagging bivalents fragment.

(6) Tetraploid progeny of triploids.

(a) C38 N.S. (2n = 24), (C38 open pollinated). Plate XIII, figs. 63-65.

The material available had been fixed in Belling's Navaschin solution and sectioned.

Metaphase. Figs. 63 and 64. Twenty-six cells were examined. As in other tetraploid types two was the maximum number of quadrivalents in a single cell. The associations observed were:-

Bivalents	Quadrivalents			x^{ta} per cell	No.of cells	Total x^{ta}
$1x^{a}$	$2x^{ta}$	$3x^{ta}$	$4x^{ta}$			
12	-	-	-	12	12	144
11	1	-	-	13	7)	
10	-	1	-	13	2)	117
10	2	-	-	14	2)	
10	-	1	1	14	2)	56
8	-	-	2	16	1	16
			Totals		26	333

Average chiasma frequency per potential bivalent = 1.07

The types of configuration did not differ from those in other tetraploids.

Anaphase. Fig. 65. This stage was not studied extensively. One cell was, however, found with three lagging bivalents, one of which formed a chromatid bridge with a small fragment adjacent.

(b) C37 N.S. (2n = 24), (C37 open pollinated).

Only a very small amount of this material, in which fixation had been rather unsatisfactory, was available for examination.

Cells examined at metaphase and anaphase I and II agreed with the behaviour described above for C38 N.S. Extended analysis was not possible with the material available.

POLLEN EXAMINATION

From their behaviour as pollen parents in crosses with the tetraploid P. alpina (Edin.) and P65 it has been inferred that PsS9 and P113 must produce occasional diploid pollen grains in addition to the normal monoploid ones. Two attempts were made to estimate the proportion of such abnormal diploid pollen.

Measurement of pollen grain size. It was considered possible that the size of diploid and monoploid pollen grains might differ significantly.

The size of pollen grains was measured in three diploid and two tetraploid forms. Slight differences in average values were found in different types, but, these were neither constant nor significant. Further examination was therefore abandoned.

Results:	Average Diameter of grains.
Diploids	
P. carinata	28.28 ± 2.55 microns
P113	26.26 ± 1.6 "
P111	28.32 ± 0.65 "
Tetraploids	
P65	28.32 ± 1.1 "
C21 (P.alpina (Edin.) x PsS9)	27.7 ± 1.3 "

Chromosome number in Pollen Grains. As an alternative approach to this problem an attempt was made to determine chromosome numbers at mitosis in pollen grains. In all cases, however, the exine became too thickened before division of the nucleus and aceto-carmine failed to penetrate. Consequently the necessary preparations could not be made.

IV. DISCUSSION AND CONCLUSION.

THE SIGNIFICANCE OF CHIASMA FREQUENCY.

The object in this section is to assess how far the available cytological data indicate the degree of relationship between the chromosome complements of the different regional types of Plantago investigated. First, however, it is necessary to discuss in general terms the extent to which chromosome association at meiosis may be regarded as an indication of genetic homology between the pairing chromosomes.

The minute study of plant chromosome structure, using normal cytological technique, is only possible at pachytene when pairing has just been established. Unfortunately detailed examination can be made only in rare instances of particularly favourable material. Where accounts of such studies are available, e.g. in Lilium and Aloe, Belling (1928) and Bellevalia, Dark (1934), the pairing chromosomes were shown to be extremely elongate and to agree minutely in their chromomeric structure. In the paired condition exactly similar chromomeres were seen to be in close conjunction. This precise agreement in physical organisation between pairing chromosomes has also been demonstrated in animals, most notably in the Salivary

Gland chromosomes of Diptera. Detailed observation is greatly facilitated in this last instance by the enormous enlargement of the chromosomes which are in a paired condition analogous to that of meiotic chromosomes, e.g. Koller (1936).

It has been commonly assumed that this detailed physical resemblance of paired chromosomes is a reflection of genetic homology, an assumption which is in keeping with inferences from "cross-over" data derived from breeding experiments. Conversely it has been assumed that where pachytene pairing takes place it is promoted solely by mutual attraction between genetically homologous particles of the associating chromosomes.

From these considerations it seemed permissible to conclude that, according to the nature of the pachytene pairing, evidence might be found in hybrid plants respecting the phylogenetic homologies between the chromosomes of the parent forms.

Unfortunately detailed observation at pachytene proved to be impossible in the Plantago material. Here, as in most dicotyledons, critical examination is possible only in the later stages of meiosis. Consequently it became necessary to judge whether pairing at later stages provides a basis for inference

concerning the genetic homology of the associated chromosomes as sound as that given by pachytene pairing.

At post pachytene stages of meiosis contact between bivalent chromosomes is maintained by one or more chiasmata. These, in Darlington's commonly accepted interpretation, result from breakage and reunion between half chromosomes or chromatids derived from opposite chromosomes. The essential problem was to decide as exactly as possible the relation between chromosome pairing at pachytene and the later pairing by chiasmata.

The details of chiasma formation and incidence have been very thoroughly investigated particularly by Darlington and his co-workers. An examination of the publications of this group together with other papers enabled the following conclusions to be made.

The most important fact which emerged was that all the papers seen were in agreement that chiasma formation occurs only in regions where pachytene pairing has been previously established. Consequently as a first approximation it may be inferred that, equally with pachytene pairing, pairing by chiasmata is an indication that the associated chromosomes are genetically homologous.

It was immediately obvious, however, that the incidence of chiasmata was subject to considerable variation and that the generalisation just made did not cover all the facts. For example, in Tradescantia spp., Darlington (1929b), and Secale cereale, Darlington (1933a), Scilla italica, Dark (1934), Crepis x strain, Richardson (1935b), and in Pisum sativum, Koller (1938), occasional pairs of chromosomes appeared as unpaired univalents in late meiosis, although it was apparent from their relative proximity that they had been paired at pachytene.

Besides these extreme cases where chiasmata failed to appear considerable variation was apparent between different chromosome pairs in the same cell, between homologous bivalents in different cells, and in the chromosome complements of different material. The causes of this variation were reviewed.

The simplest position appeared in cases where the several bivalents of a complement were small and approximately similar in size, e.g. Crepis capillaris and C. tectorum, Richardson (1935a), and in diploid Hyacinth spp., Stone and Mather (1932). Here the number of chiasmata per bivalent was proportional to the respective lengths of the different chromosome pairs. It is postulated that this follows

an approximately equal degree of pachytene pairing in all bivalents. In different cells the number of chiasmata formed by a given bivalent might fluctuate as a result of random variation in the completeness of pachytene pairing.

In contrast to the behaviour outlined for these small chromosome forms is the behaviour found in some species of Fritillaria, Darlington (1935), and Allium, Emsweller and Jones (1935), and Levan (1933). These two genera have very large chromosomes but in some species pachytene pairing is restricted to a short segment of each bivalent and the chiasmata are consequently strictly localised. In the extreme case each bivalent forms only a single chiasma. The localisation was shown by Emsweller and Jones to result from a definite gene mutation restricting the degree of pachytene pairing.

In cases where bivalents of a complement showed considerable discrepancy in size the number of chiasmata per unit length of chromosome differed greatly in different bivalents. For example in the Orthopteran insects, Stenobothrus, Darlington and Dark (1932), and Chorthippus and Stauroderus, Darlington (1936), as well as in plant material, Scilla italica, Dark (1934), and Eremurus spectabilis, Upcott (1936a), very long chromosomes formed a

disproportionately low number of chiasmata compared with smaller chromosomes. This behaviour, it is suggested, results from gene mutation working to secure regular pairing of the shortest chromosomes in a complement of very variable length. The property appears to be confined to stable genotypes since newly originated chromosome fragments were found in Tradescantia, Darlington (1929b), and in Matthiola, Philp and Huskins (1931), to have a chiasma frequency only equal to or less than that required by their proportionate length.

Other instances in which genetic factors were supposed to result in abnormal chiasma frequency were in Crepis x strain, Richardson (1935b), and in an asynaptic line of Pisum, Koller (1938). In both cases the total number of chiasmata per cell was considerably lower than in samples of material from closely related lines. The position with regard to chromosome pairing was summarised as follows. Chiasmata are found only after pachytene pairing, and association by chiasmata may therefore be regarded as evidence that the paired chromosomes are genetically homologous. Any estimate of the degree of genetic homology from the frequency of chiasmata must, however, be made with care.

The formation of several chiasmata in any bivalent is a good indication of homology but a low chiasma frequency or failure to form chiasmata may proceed from several causes, in addition to genetic non-homology reducing or prohibiting pachytene pairing. To safeguard inference the following precautions were considered advisable -

(1) A statistical estimate of chiasma frequency should be made from the largest possible sample.

(2) In the case of a hybrid an estimate of chiasma frequency should be made from both parents for comparison with that of the hybrid.

(3) Careful consideration of any special conditions attaching to each particular case.

By exercising these precautions it was felt that a reasonably sound estimate might be made of the relative homology of the chromosome complements of related lines.

In the Plantago material studied it is apparent that low chiasma frequency prevails throughout. Normally each chromosome forms one chiasma, though fairly frequently two may appear. Only in the triploid types was a single chromosome seen to participate in three chiasmata.

The causes underlying this low chiasma frequency could not be directly analysed. Because of observational difficulties it was impossible to decide whether pachytene pairing was restricted to a segment of bivalents or whether the small size of chromosomes directly limited the chiasma frequency.

A comparative analysis was made for all diploid and tetraploid types in order to find whether chiasma frequency varied significantly in the material studied.

Diploid types. All the types studied within this category, P. carinata, P. serpentina, P82 and the hybrids C28 and C29, showed a marked similarity of behaviour. Six bivalents were invariably formed and failure of pairing was never observed. With a maximum frequency of two chiasmata per bivalent and of two such bivalents per cell the maximum chiasma frequency was 1.33 per bivalent. Since bivalents normally formed a single chiasma the minimum chiasma frequency in any cell was 1.0 per bivalent. For the lines examined the chiasma frequencies lay between 1.03 and 1.12.

The five diploids were comparable as far as the proportion of bivalents forming two chiasmata were concerned. The average frequency of ring bivalents

TABLE III.

TABLE III.

Examination of relative frequency of one and two chiasma bivalents by chi-square test.

Type	No. of cells	Observed No. of Bivalents		Total Bivalents	Expected No. of Bivalents		Deviations from expected		Chi-square	Probability
		1 chiasma	2 chiasmata		1 chiasma	2 chiasmata	1 chiasma	2 chiasmata		
Diploids										
P.carinata	128	717	51	768	693.5	74.5	+23.5	-23.5	8.2	0.01
P.serpentina	23	121	17	138	124.5	13.5	- 3.5	+ 3.5	1.006	0.50-0.30
P82	20	108	12	120	108.4	11.6	- 0.4	+ 0.4	0.0155	0.90
C28	125	662	88	750	677.3	72.7	-15.3	+15.3	3.5671	0.10-0.05
C29	110	593	67	660	596.0	64.0	- 3.0	+ 3.0	0.1561	0.70-0.50
Totals	406	2201	235	2436	2199.7	236.3	+ 1.3	- 1.3	0.0078	
					Total chi-square (4 deg. freed.)				12.9368	0.02-0.01
					Total chi-square, neglecting P.carinata (3 deg. freed.)				4.7458	0.20-0.10
Tetraploids										
P63	26	287	25	312	281.7	30.3	+ 5.3	- 5.3	1.0267	0.50-0.30
P.alpina (Edin.)	54	575	73	648	585.1	62.7	-10.1	+10.1	1.796	0.20-0.10
C21	23	247	29	276	249.2	26.8	- 2.2	+ 2.2	0.2000	0.70-0.50
C22	12	126	18	144	130.0	14.0	- 4.0	+ 4.0	1.266	0.30-0.20.
C38 N.S.	26	291	21	312	281.9	30.3	+ 9.3	- 9.3	3.162	0.10-0.05
Totals	141	1526	166	1692	1527.9	164.1	- 1.9	+ 1.9	0.024	
					Total chi-square (4 deg. freed.)				7.4267	0.10-0.05
					Total chi-square for 2X and 4X (neglecting P.carinata 9 deg. freed.)				11.9043	0.30-0.20

Average freq. of two chiasma bivalents

$$= \frac{235 + 166}{2436 + 1692} \times \frac{100}{1}$$

$$= 9.7\%$$

in the diploids was 9.64% compared with an average value for all diploid and tetraploids of 9.7% (Table III). For each diploid the deviation was calculated of the observed value from that expected with 9.7% of ring bivalents. The significance of the deviations was tested by means of the X^2 (chi-square) test (Fisher, 1936). Only P. carinata showed a significant deviation in the number of two chiasma bivalents observed from that expected. Apart from this type all the diploids formed a homogeneous group in respect of chiasma frequency, the total chi-square (4.7458) having a 10-20% probability. The cause of the significantly lower frequency in P. carinata is unknown, it may possibly be genetical. As regards the genetic homology of the diploid types the behaviour of the hybrids C28 and C29 indicated a high degree of homology between the parental forms P. carinata, P. serpentina and P84.

Tetraploid types. The forms investigated were of four different types.

(a) P. alpina (Edin.))
P63) Parental types.
P65)

(b) C22 Hybrid from cross P. alpina (Edin.) x P63 (i.e. tetraploid x tetraploid).

(c) C21 Hybrid from cross P. alpina (Edin.) x PsS9 (i.e. tetraploid x diploid).

(d) C38 N.S. From the open pollination of the triploid C38.

These types also showed marked similarity in behaviour. The chromosome association within a single cell varied in each type between twelve bivalents and ten or eight bivalents with respectively one or two quadrivalents. As in the diploids, bivalents had normally one, occasionally two chiasmata. The limits of chiasma formation in any cell were between 12.0 when twelve rod bivalents were present (average 1.0 chiasma per bivalent) and 16.0 when eight rod bivalents were accompanied by two ring quadrivalents, or by one such quadrivalent with two ring bivalents (average 1.33 chiasmata per potential bivalent). The extremes of chiasmata formation are, therefore, exactly comparable in both diploids and tetraploids.

The average frequency of bivalents, or potential bivalents, with two chiasmata was 9.8%; as in the diploids the expected values and their deviations from the observed values were calculated for the general average of 9.7% (Table III, page 59). The deviations were not significant for any type when tested by chi-square. The summed chi-square for all the tetraploids was not significant, showing that they form a homogeneous group in respect to chiasma formation.

In order to compare the tetraploids and diploids the individual chi-squares for all types investigated (except P. carinata) were summed. The total, 12.6073, has a 20-30% probability. It may, therefore, be concluded that chiasmata formation is comparable in these five diploid and five tetraploid types.

Although there is no significant difference between diploids and tetraploids it may be pointed out that the average chiasma frequency per bivalent for all tetraploids (1.098) is slightly lower than the comparable figure for diploids (1.11) (excluding P. carinata). This behaviour is in keeping with that generally found in related tetraploid and diploid types (Upcott, 1939).

The chromosome pairing in C22 indicates the same degree of genetic homology between the complements of P. alpina (Edin.) and P63 as exists between the haploid sets of either parent. The significance of the behaviour observed in C21 and C38 N.S. will be considered later.

Triploid types. The types investigated, C37, C38 and C39, represent crosses of the tetraploid P. alpina (Edin.) with various diploid forms.

In all cases several trivalents were formed, the maximum number of six being seen in three cells of C38, five being the most found in C37 and C39. The maximum number of chiasmata per cell in these triploids was fourteen in C37 (Plate X, fig. 49, page 40). In the cells of C38 with six trivalents thirteen chiasmata were found, five of the trivalents each having two chiasmata whilst the sixth had three. The maximum number of half-chiasmata per chromosome in these types was 1.44-1.55, this being higher than the comparable maximum chiasma frequencies in the diploid and tetraploid forms. This behaviour is in keeping with the higher relative chiasma frequency observed in other triploids, e.g. Hyacinthus, Stone and Mather (1932), Tulipa, Darlington and Mather (1932).

The discrepancy between tetraploids and triploids is probably due to the fact that each chromosome is likely to achieve a higher degree of pairing where three rather than four chromosomes are competing at pachytene.

STRUCTURAL HYBRIDITY (INVERSION)

The term "inversion" as used in this paper denotes a condition in which part of a chromosome thread has become inverted with respect to the centromere.

As already mentioned in the account of the Plantago observations the presence of an inversion in one chromosome of a bivalent is recognised by the appearance of a bridge chromatid at late anaphase. Such a bridge follows a special type of pachytene pairing. In an individual heterozygous for an inversion, homologous pairing is achieved by "reverse" or "loop" pairing within the inverted region provided this is sufficiently long. If a chiasma is formed in the inversion loop, a dicentric chromatid, connecting the two centromeres of the bivalent, and an acentric chromatid result, (see Darlington, 1937, pp. 266-7, fig. 90, 91.). The remaining non-crossover chromatids are respectively one normal and one inversion chromatid. At anaphase the dicentric chromatid remains for some time as a "bridge" across the equator between the centromeres moving to opposite poles. The acentric fragment does not segregate and is usually excluded from the nuclei resulting from

meiosis. This characteristic anaphase bridge has been found, and inversion heterozygosity therefore inferred in several cases (e.g. Crepis divaricata x C. dioscoridis, Muntzing (1934); Crepis Capillaris x Strain, Richardson (1935); Lilium martagon album x L. Hansonii, Richardson (1936); Paeonia spp., Dark (1936); P. suffruticosa Sax (1937); Fritillaria spp., Frankel (1937); Tulipa spp., Upcott (1937)). The bridge may break during first anaphase or may persist and be found joining two nuclei of the tetrad. In any event the two nuclei of the tetrad which receive parts of the bridge will be deficient through the absence of the acentric fragment. Such nuclei are probably in most cases non-viable. The remaining two nuclei in the tetrad each receive a non-crossover chromatid, one the normal, the other the "inversion" chromatid. Hence of the fifty per cent viable nuclei resulting after crossing over in an inversion loop, half carry the unchanged inversion, whereas no viable nuclei carry cross-over products between the inversion and its homologous normal region.

Richardson (1936) considered the configurations expected where two chiasmata were formed in the inversion loop. The results of crossing-over depend on the relationships of the successive chiasmata

of which there are three possible:

(a) Reciprocal - involving the same two chromatids.

(b) Disparate - one chromatid forming chiasmata at different points with two other chromatids.

(c) Complementary - a different pair of chromatids associating at each chiasma.

The genetical consequence compared with those following a single chiasma in the inversion are similar in that two deficient spore nuclei result from each dicentric bridge but differ in that from the reciprocal and disparate chiasmata viable nuclei may arise carrying gene re-combinations between the inversion and its homologous non-inverted region.

It has further been shown, Frankel (1937) and Upcott (1937), that the stage at which the chromatid bridge becomes obvious depends on the number and relationship of any chiasmata formed between the centromere and any chiasmata in the inverted region. Thus a proximal chiasma disparate to a single chiasma in the inversion loop results in a dicentric chromatid loop at first anaphase which becomes a "bridge" at second anaphase. These authors considered the results of all possible combinations of chiasmata proximal to an inversion containing one or two chiasmata. The only change resulting is a conversion of first to second anaphase bridges or vice versa; there is no influence on the number of dicentric chromatids.

In Plantago inversion hybridity was inferred from observation of anaphase bridges in the tetraploid P. alpina (Edin.) and several hybrids related to it, notably C21, C22, C38, C39 and C38 N.S. Apart from the types in this group bridges were observed at first and second anaphase in P. carinata and a single case of a bridge chromosome at second anaphase in P84 (see Plate IV, fig.15, page 23).

The inference, that anaphase bridges arose from crossing over in heterozygous inversions, is made with considerable certainty in P. alpina (Edin.)and its related forms, where the expected acentric fragment was observed in several cases. In P. carinata, although a bridge was seen in several cells, no accompanying fragment was found. Inversion hybridity is therefore inferred here with some uncertainty, although the possibility is strengthened by observation of a bridge at both anaphase I and II. It is unfortunate that the pachytene stage could not be analysed in P. carinata. This might have shown whether, in fact, inversion loop pairing occurred at pachytene.

The impossibility of observing stages earlier than diakinesis was unfortunate in another direction. The bridges observed at second division in P. carinata and C39, and the double bridge of one bivalent in C21 require at least two chiasmata in one arm of the

bivalent if they arise from inversion heterozygosity. For an anaphase II bridge one chiasma must occur in the inversion loop and proximally, a second disparate chiasma. Two complementary chiasmata in the inversion loop are required for the double anaphase I bridges. At diakinesis and metaphase in normal bivalents more than one chiasma has never been observed on the same side of the centromere; ring bivalents always had the two chiasmata on opposite sides of the centromere. It is possible that more than one chiasma may be formed in the same arm of the chromosome in a normal bivalent. If so, all except one chiasma must be supposed to disappear by terminalisation. This is improbable. Occasional observations of two chiasmata in one arm even at metaphase would be expected in such a case.

It may be pointed out, however, that in the triploids two types of trivalent were found with a triple chiasma which must result from two chiasmata forming in one arm of the chromosome. It is possible that with an inversion paired in one arm special conditions of stress may arise, not present in normal bivalents, resulting occasionally in a second chiasma.

The inversion heterozygosity seen in P. alpina (Edin.) and the group of related hybrids

probably derives from inversion in the common parent, P. alpina (Edin.). It seems likely that one pair of chromosomes here is homozygous for an inversion. Normally they probably pair together but occasional pairing between the inversion chromosomes and an homologous pair, lacking the inversion, must take place. Anaphase bridges would result from the heterozygous pairing.

Occasionally from such a pairing two inversion chromosomes might enter a single anaphase nucleus. The presence of two inversion chromosomes was indicated in C21 by the occurrence of two separate anaphase I bridges (Plate VIII, fig. 39, page 34). These probably both derived from the egg nucleus of P. alpina (Edin.) since no inversion hybridity was found in the other parent of C21, namely the diploid PsS9, or in its diploid hybrids C28 and C29.

The frequency with which inversion crossing over is observed varies greatly in different material. Thus Frankel (1937) found inversion crossing over in about 60% of pollen mother cells in diploid Fritillaria dasyphylla, whilst Upcott found in Tulipa some types with more, some with less, than 10% of bridges, and in Aesculus hippocastanum (1936) she found 2-3%, whereas in Fritillaria chitralensis

Bennett (1938) found only 0.7% of the cells with bridges. Speaking broadly one may say that these differences arise from different proportions of inverted/non-inverted lengths in the chromosomes. Long inversions will more frequently act independently to form inversion loops at pachytene and these will have a proportionately high chiasma frequency. Short inversions on the other hand will, for mechanical reasons, not usually form inversion loops at pachytene. They will have difficulty in behaving independently and will usually continue, by torsion, the linear homologous pairing of the adjacent non-inverted regions, McClintock (1933), Darlington (1936). For this reason, and because in any event the incidence of chiasmata in the short inversion will be rare, crossing over in the inversion will be infrequent. Darlington (1936, 1939) has emphasised the distinct genetical results which follow the occurrence of these long and short inversions in living populations.

Where the inversion is homozygous meiosis is normal and, provided there is no natural selection against any genetical result of inversion, the homozygous progeny will survive and multiply.

In hybrids, between inversion and normal lines, it has been shown that the inversion is not

completely destroyed. The results of crossing over between the inversion and the homologous normal region are lost in the 50% of all spores which are non-viable as a result of one or two chiasmata within the inversion. With one or two inversion chiasmata, 25% and 8.3% represent the minimum respective proportions of all the spore nuclei which carry the unchanged inversion. The maximum proportion of spores carrying viable cross-overs within the inversion is 33.3%, where two chiasmata occur at random amongst the four chromatids.

With respect to the occurrence of viable spores carrying inversion crossovers it should be emphasised that the figure of 33.3% represents a maximum. It is to be expected that, fairly frequently, where two is the maximum, fewer chiasmata will occur inside the inversion and this will reduce the percentage of viable recombinations. This follows from

(1) The normal fluctuation in number and incidence of chiasmata largely dependent on variation in pachytene pairing.

(2) Darlington's finding (1936) that the incidence of inversion chiasmata was considerably less than expected from the proportionate length of the inversion measured at pachytene.

(3) The general finding that chiasma frequency is

lower in an inversion heterozygote than in the comparable homozygote. Richardson (1936), for example, found that Lilium martagon album had an average frequency of 4.07 chiasmata per bivalent, whereas its hybrid with L. Hansonii, showing inversion heterozygosity besides other structural change, had only 2.19 chiasmata per bivalent.

Such inversion heterozygotes, with frequent crossing over in the inversion, show a marked sterility. Thus, diploid tulips showing more than 10% of anaphase bridges were found by Upcott (1937) to have a high proportion of bad pollen and to set seed only rarely; their successful survival depending on vegetative reproduction. The inverted region in such forms will be unstable being subject to destruction through chiasma formation. Moreover, although recombination between genes in the inversion and their homologues is limited, such gene exchange is possible wherever more than two inversion chiasmata are formed. Long inversions may not therefore be considered as an isolating mechanism of any great evolutionary importance in a sexually reproducing population.

A sharp contrast is found in plants heterozygous for very small inversions. As has already been indicated the incidence of chiasmata in such

inversions in the heterozygote will be very low. At most, a single inversion chiasma may occasionally be formed and this will probably not result in any viable cross-over products. The viability will, doubtless, depend on the length of the acentric fragment which determines the degree of deficiency. In any event, the genes in such short inversions will approach a state of total linkage. Any mutated genes which arise will therefore be inherited with non-mutated genes in the inversion as a block characteristic of the inversion. In time they may thus become a focus of genetic discontinuity isolated from recombination with the gene complex of the general population. Darlington has termed them 'floating inversions', emphasising that, for every one detected by occasional chiasma and bridge formation, several others probably exist in any population.

As regards the genetical results of inversion in the Plantago material no direct evidence is available. By analogy with Darlington's suggestions outlined above it may be inferred that the genes present in the inverted segments will be strongly linked.

The extent to which any inversion race develops under field conditions will depend on the

relation between environmental factors and any characteristic genetic properties which the inversions may acquire. Sturtevant and Mather (1938) are of the opinion that mutations occurring in inversions will probably yield recessive lethal genes. Such inversions are likely to persist only in the heterozygous state. It is conceivable, however, that inversions may acquire genes which prove advantageous under natural selection. In such cases inversion races may develop on lines divergent from the rest of the population. To what extent this development has occurred in Plantago it is impossible to say.

INTERRELATIONSHIPS OF DIPLOIDS AND TETRAPLOIDS.

The behaviour at meiosis in polyploid plants is very variable, extreme types being represented by true auto- and allotetraploids — formed respectively by doubling the chromosome complement in fertile and sterile forms, Kihara and Ono (1926).

In the autotetraploid four homologous chromosomes of each type are in the spore mother cell nucleus. It was found by Newton and Darlington (1929) and Darlington (1929, 1931a) that pachytene pairing takes

place only between two chromosomes at any one point. At other points the partners of the original pair may separate and become paired with a third or fourth chromosome. Each chromosome may at different points pair with any or all of its homologues. By this pairing in twos, with exchange of partner, all four chromosomes may be associated in a single configuration. Subsequently chiasmata are formed between non-sister chromatids in regions where a sufficient degree of pachytene pairing has previously been attained between any two chromosomes.

Subsequently pairs of chromatids fall apart at diplotene except where chiasmata have been formed between them. In the absence of chiasmata between any two chromosomes they become entirely separate at diplotene and remain so henceforward. Thus at these stages may be found quadrivalents, with all four homologous chromosomes associated by chiasmata, trivalents with univalents, or bivalents.

The factors which appear most important in affecting pachytene pairing are -

(I) Competition between homologous chromosomes.

(II) Relative position of chromosomes.

(III) Size of chromosomes, speed of pachytene pairing being inversely proportional to length.

Competition arises because close pairing association between two chromosomes at one point tends to exclude other partnerships in adjacent sections. Pairing established in one segment appears to extend because mutual attraction between the chromosomes in regions adjacent to the paired sector is greater than the attraction between either chromosome and a third. Pairing takes place, therefore, in autopolyploids by random association, not of individual chromomeres but of considerable sections of chromosomes which pair completely. This behaviour was described in Tulipa, Darlington and Mather (1932), as pairing by random association of a small number of "pairing blocks" in each chromosome.

The interaction of these factors may be seen in the results from autotriploid Hyacinthus, Stone and Mather (1932). Here it was found that the chiasma frequency per unit length was greater in chromosomes of medium length (9 micron), than in longer (21 micron), or shorter ones (5 micron). The observations of chiasma frequency and trivalent formation were in agreement with the hypothesis that the medium chromosomes paired by random association of ten pairing blocks in each chromosome, whereas the short and long chromosomes had only five and two pairing blocks

respectively. The small number of pairing blocks in the long chromosomes arises from their relatively excessive length which results in slow movement and incomplete pachytene pairing before chiasmata are formed. The shortest chromosomes suffer because of their small absolute length which reduces the possibility of sufficient pairing with exchange of partner to allow chiasma formation between more than two homologous chromosomes, although they must be supposed to move more freely than the larger chromosomes.

As in the bivalents of diploid organisms, so in multivalent configurations terminalisation of the chiasmata may or may not take place. Where it occurs the chromosomes in multivalents remain associated only by their ends in relatively simple configurations. Darlington (1931a) has shown that in these circumstances four types of trivalents and ten types of quadrivalents may be expected, according to the number and incidence of chiasmata. All these configurations have been found in Datura stramonium or Primula sinensis (vide Darlington 1931a).

Where the chiasmata are not terminalised the multivalent configurations at metaphase are more variable and complex in form.

Metaphase orientation and anaphase separation

may show considerable variation in the autotetraploid according to the configurations formed at earlier stages. Any bivalents which occur behave entirely as in normal diploid meiosis. Quadrivalents however, become variously orientated at metaphase, according to the number and position of chiasmata. Thus a chain quadrivalent or a simple ring may have adjacent chromosomes on opposite or on the same sides of the equator. Other types with terminal chiasmata are unable to achieve any symmetrical orientation and this is true also of quadrivalents with interstitial chiasmata. Subsequently two of the four chromosomes may pass to each pole but an unequal 3:1 distribution may occur. This unbalanced distribution was found in Primula sinensis by Darlington (1931a) most frequently in quadrivalent types 11, 12, 13 and 14 of his classification, Darlington (1937, p.124, fig.40) that is, where several free ends occur. In some cases, also, one chromosome may remain on the equator after the segregation of the other three. The trivalent is inherently irregular, a 2:1 distribution of the chromosomes being inevitable if they all segregate, otherwise one must be left on the equator. Univalents present before metaphase do not usually congress on the plate with the paired chromosomes, but move at

anaphase on to the equator. Here, along with any chromosomes left from the segregated multivalents, they move to either pole intact, or divide as at mitosis, the halves passing to opposite poles. The behaviour of univalents is, however, quite uncontrolled. They are not necessarily distributed to opposite poles in equal numbers and they may fail to be included in either of the polar nuclei.

As a consequence of these anaphase irregularities it is frequently found that in the first division of the polar nuclei the number of chromosomes differs from the normal diploid number. The spore nuclei arising after second division may therefore contain the diploid complement or some slightly different number of chromosomes. Thus Upcott (1935) found in tetraploid Lycopersicum esculentum that only 63% of the gametes had the diploid chromosome number of n = 24, whilst 37% had chromosome numbers of 22-26. She quotes two other investigations of similar material where 19.1% and 22.6% of unbalanced gametes were found.

Such aberrant spores may give rise to functional gametes, where the deviation from a balanced diploid complement is not too great. The progeny of an autotetraploid may therefore consist of individuals

having the full tetraploid, or a somewhat different chromosome number. Too great a departure from the diploid number in the spore nucleus results in a serious state of unbalance, so that the spores or the gametes are not functional. It must also be pointed out that irregular segregation of univalents may result in nuclei containing an approximate or complete diploid number of chromosomes, but unbalanced genetically from unequal distribution of homologous chromosomes. The deficiencies are reciprocal in the two polar nuclei and consequently all the spores of the tetrad will be non-functional.

Contrasting strongly with the behaviour outlined for autotetraploids is that of the extreme allotetraploid. The classical example is Raphanus-Brassica, Karpechenko (1927). A hybrid was obtained between Raphanus sativus (2n = 18) and Brassica oleracea (2n = 18) which possessed a diploid complement of eighteen chromosomes and was almost completely sterile. At meiosis it was found that no pairing took place, eighteen univalent chromosomes being present. At first division these either segregated at random or remained as a group of eighteen. The spore nuclei were therefore formed from variable numbers of chromosomes up to a maximum of eighteen.

Most of the spores were non-functional and only a very limited second generation progeny was obtained. Among these were a few tetraploid plants which had arisen from the fusion of two gametes carrying the full complement of eighteen chromosomes. Meiosis was perfectly regular only bivalents being formed by pairing together of homologous Raphanus or Brassica chromosomes. No "interspecies" pairing between Raphanus and Brassica chromosomes occurred. The tetraploid was therefore highly fertile, producing a uniform tetraploid progeny.

Thus in respect of meiotic behaviour in tetraploids a broad distinction may be drawn between

(1) Autotetraploids containing four sets of homologous chromosomes forming at meiosis uni-, tri- and quadrivalents which by their irregular behaviour result in some degree of sterility.

(2) Allotetraploids containing two differentiated diploid complements. Each chromosome has only a single homologue; consequently bivalents are always formed at meiosis and the plants have full fertility.

In tetraploids generally, however, meiosis may present all degrees of variation between the extremes. The formation of configurations other than bivalents is conditioned primarily by chromosome

homology, secondarily by the circumstances, genetical and mechanical, affecting chiasma formation.

An example of intermediate tetraploid meiotic behaviour is the Cambridge Raphano-Brassica material, Howard (1938). The original diploid hybrid differed from Karpechenko's F_1 in having 1-6 bivalents plus 16-6 univalents at meiosis instead of eighteen univalents, indicating that homologies existed between some of the Raphanus and Brassica chromosomes. Meiosis in the derived tetraploid bore out this idea - a proportion of tri- and quadrivalents being formed. Howard found that some of the pairing chromosomes were heterozygous for inversions and he suggested that they were probably also not entirely homologous genetically. Segregation may therefore result in variable and sometimes unbalanced genetical combinations, while numerical variation may result from irregular segregation of multivalents and univalents. The tetraploid therefore shows some sterility and genetical and cytological variation in the progeny.

Tetraploid Primula Kewensis, Upcott (1939), illustrates another type intermediate between the characteristic auto- and allopolyploid behaviour. This plant has originated as a fertile shoot on three separate occasions, by somatic doubling, from a sterile

diploid hybrid between P. floribunda (2n = 18) and P. verticillata (2n = 18). The parental species are fully fertile, forming nine bivalents at meiosis with average frequency of 2.0 and 2.13 chiasmata per bivalent in P. floribunda and P. verticillata respectively. The diploid hybrid also frequently forms nine bivalents at meiosis although two or four univalents may often be present. The average chiasma frequency in the hybrid is, however, only 1.39 per bivalent. Thus, although the chromosomes of one parent are sufficiently homologous to pair with those of the other, the markedly reduced chiasma frequency indicates that the homology is by no means complete. The reduced chiasma frequency may be accounted for in part by inversion heterozygosity, detected by fairly frequent anaphase bridges. Considerable genetic differentiation between pairing chromosomes was also postulated. Gametes containing the normal monoploid number (9) of chromosomes are often non-functional because it was suggested they may represent combinations of P. floribunda and P. verticillata chromosomes which are genetically unbalanced. In addition to these sources of sterility, random segregation of univalents resulted in non-functional pollen nuclei with 7, 8 or 10 chromosomes instead of

the normal 9.

The tetraploid plant at meiosis formed on the average 12.4 bivalents, 0.4 trivalents with univalent and 2.4 quadrivalents. The average chiasma frequency was 1.90 per potential bivalent, representing 0.92 of the combined average chiasma frequencies of the diploid parents. The formation of a majority of bivalents in this tetraploid illustrates competition in pairing. The purely mechanical factors underlying competition were discussed for autopolyploids but in Primula Kewensis genetic and structural differentiation of the chromosomes must also be considered.

In the diploid hybrid, chromosomes of one parent form bivalents with partially homologous chromosomes of the other, although with reduced chiasma frequency. In the tetraploid fully homologous floribunda chromosomes can pair and verticillata chromosomes similarly. Any tendency to form configurations with "interspecific" pairing apparently fails, in most cases, because the attractions between fully homologous chromosomes are stronger than between partially differentiated ones. Multivalents necessitating "interspecific" association are therefore only formed in about 30% of the complement. Consequently the tetraploid has a high degree of

constancy and fertility, the only sources of variation being the cross over products from "interspecific" pairing and irregularity in the segregation of multivalent configurations.

In Plantago the meiotic behaviour observed individually in P. alpina (Edin.), P63 and P65 does not permit of any conclusion regarding their allo- or autotetraploid constitution. The chromosome pairing observed is intermediate between that expected for extreme types of allo- and autotetraploid. The formation of one or two quadrivalents in these types does indicate that two distinct sets of chromosome pairs are sufficiently homologous to allow of pachytene pairing and chiasma formation. Because of the lack of visible differentiation between chromosomes it is impossible to determine whether the same chromosomes always form the quadrivalents. Therefore, the relative homology of eight pairs of chromosomes cannot be directly determined.

It may be pointed out, however, that all diploids examined, whether population samples or hybrid types, have shown regular meiosis. Gregor has also found that these, and in addition many other diploid forms, are highly fertile. There does not, in fact, appear to be any differentiation amongst the diploid

chromosome complements such as would be necessary for the production of allotetraploids. It may, therefore, be presumed that the forms in question are autotetraploids.

In this connection the behaviour of C21 (tetraploid P. alpina (Edin.) x diploid P. serpentina) provides some evidence. This plant must contain one diploid complement of P. alpina and one diploid complement, presumably from a diploid pollen grain, of P. serpentina. It is reasonable to suppose that intra-monoploid pairing does not take place in any of the Plantago material and consequently two methods of chromosome pairing are possible in C21. The twelve P. serpentina chromosomes may form six bivalents and, in that event, the P. alpina chromosomes must also form six bivalents. Alternatively, the twelve P. serpentina chromosomes may pair with the twelve P. alpina chromosomes to form twelve bivalents. Quadrivalents must represent associations of the two P. serpentina with two P. alpina chromosomes. Pairing in the former case could only occur if the twelve P. alpina chromosomes comprise two homologous sets of six. In the second case, it may also be inferred that six homologous pairs of P. alpina chromosomes are present since they pair with two homologous monoploid sets of

P. serpentina. It may therefore be concluded that P. alpina (Edin.) is an autotetraploid.

Additional evidence in support of this conclusion may be drawn from the triploid hybrids of P. alpina (Edin.), namely C37, C38, and C39. As reported above these forms may all show a number of trivalents, the maximum of six having been observed in C38; wherever trivalents are formed two P. alpina chromosomes must either pair together directly or through the intermediacy of a third chromosome from the diploid parent, such behaviour indicating a high degree of homology between the two sets of six chromosomes in the haploid complement of P. alpina (Edin.).

The behaviour of the tetraploid hybrid C22 (P. alpina (Edin.) x P63) provides a basis for inferring the constitution of the tetraploid P63. Chromosome association in this hybrid is perfectly comparable with that of P. alpina. It may therefore, be argued that the chromosomes of P63 either pair together or with those of the known autotetraploid, P. alpina. P63 must, therefore, be autotetraploid.

STABILITY AND FERTILITY OF TETRAPLOIDS.

The persistence of polyploid lines in the field will naturally depend to a great extent on the degree of fertility they show. Experimental evidence favours the view that allopolyploids are likely to have full fertility whereas autopolyploids are likely to be partially sterile due to meiotic irregularities following the formation of multivalents. Muntzing (1936) points out, however, that natural autotetraploid types very frequently have a higher fertility and constancy than has been found in experimental forms. In agreement with Darlington (1937) he supposes that under prolonged natural selection survivors of original autopolyploids may have chromosome sets differentiated by structural change and consequently with regular meiosis and high fertility. In connection with the same problem, Kostoff (1939) has drawn attention to the high frequency of small chromosome types in natural polyploids. The small chromosomes with low chiasma frequency form few or no multivalents and the polyploids are therefore highly fertile, even though, in origin they may be autopolyploid. In polyploids where multivalents are formed at meiosis a proportion of aberrant spores may function besides the normal spores, provided that the degree of unbalance is not

too great. Variation amongst the progeny is then inevitable. Thus in the tetraploid Biscutella laevigata (2n = 36) Manton (1937) found about 20% of the progeny had 34, 35 or 37 chromosomes instead of 36.

Assuming then that the plantain tetraploids are autopolyploids it might with reason have been expected by analogy with the above observations, that their fertility would be less than that of their diploid relatives. Contrary, however, to this expectation Gregor found that the numbers of capsules set and the numbers of seeds present in each capsule were as high for P. alpina (Edin.) growing under greenhouse conditions as in any of the European diploids. Similarly, both the directly obtained tetraploid hybrids (C21 and C46) from the respective crosses P. alpina (Edin.) tetraploid x P. serpentina diploid and P113 diploid x P65 tetraploid and the derived tetraploids, C37 N.S. and C38 N.S.), arising from the almost completely sterile triploids, C37 and C38, were fertile to a high degree. The wild tetraploids P63, P65 and P117 also exhibited about normal diploid fertility when grown under glass and hand-pollinated. Under garden conditions, however, Gregor finds that the relative fertility of diploids and tetraploids was

less easily estimated. The flowering period of the latter was later and more prolonged than that of the diploids, and in consequence tetraploid seed production tended to decline as the season advanced. But notwithstanding the lower fertility of late spikes the average seed production of tetraploid plants was little less than that of diploids of similar geographic source. The fertility of the tetraploids seems almost certainly to be associated with the low frequency of chiasmata and quadrivalent formation. It has been shown above that chiasma formation is comparable in all the investigated diploid and tetraploid types. Because the average chiasma frequency is low the number of multivalents formed in any of the tetraploids is small. As a result meiotic segregation is regular, and in the majority of cells twelve chromosomes pass to each pole at first telophase. The plants are therefore fertile. The behaviour is in keeping with the conclusions of Kostoff (1939) concerning the fertility of polyploid plants with low chiasma frequency. This conclusion is supported by the types of quadrivalents observed. Only chains and rings of four chiasmata have been found. Types requiring a greater number of chiasmata have not been seen and would not be expected with the low chiasma frequency of these tetraploids.

From the above remarks it may therefore be safely inferred that these apparently autopolyploid populations are not only capable of successful sexual reproduction, but that in so far as seeding rate determines competitive survival in nature they are at least as favourably situated as their diploid associates.

LIMITATION OF GENE EXCHANGE.

In many genera gene exchange between related diploid and tetraploid populations, even though exchange may be potentially possible must occur very infrequently under natural conditions; for instance in the genera Agrostis (Sokolovskaya, 1938), Deschampsia (Hagerup, 1939) and Alopecurus (Strelkova, 1937) it has been found that differences in their ecological requirements effectively separate diploids from their related tetraploids. Where, however, polyploids occur with their diploid progenitors, as they must all do originally, or where their distributions adjoin or overlap, free gene exchange is limited by barriers of incompatibility and sterility.

Incompatibility arises because monoploid pollen from the diploid often fails to fertilise the

ovules of related polyploids and vice versa. Muntzing (1936) mentions many cases in which crosses of diploid x tetraploid and the reciprocal cross produce no viable seeds or only very few. From analysis of data from several authors he concludes in agreement with Watkins (1932) that incompatibility is a result of departure from the normal chromosome number relationships of pollen grain, stylar tissue, zygote and endosperm. In diploid forms this number ratio is 1n:2n:2n:3n and in tetraploids 2n:4n:4n:6n, whereas in diploid x tetraploid it is 2n:2n:3n:4n and reciprocally 1n:4n:3n:5n.

In the occasional instance where viable progeny do result from crosses between related diploid and tetraploid lines they usually have the triploid chromosome number. Where these are autotriploid the homologous chromosomes may associate as trivalents or as bivalents and univalents. The proportion of chromosomes as trivalents varies from cell to cell in any material with random variation in the length of pachytene pairing and chiasma formation. Thus in triploid Zea mays (n = 10) McClintock (1929a) found from ten trivalents to six trivalents with four bivalents, and in triploid Lycopersicum (n = 12) Upcott (1935) found 3-7 trivalents with 9-5 bivalents and

univalents per cell. Triploids derived from an extreme allotetraploid crossed with a related diploid type do not form trivalents. The maximum association at meiosis is n bivalents and n univalents, the monoploid chromosomes set from the pollen pairing with half the complement of the tetraploid. Such behaviour is shown by new world cultivated Gossypium (2n = 52) crossed with wild American species (2n = 26). The triploids (2n = 39) approximate to the scheme of 13 bivalents plus 13 univalents, Skovsted (1937).

In any event triploids are inherently irregular at anaphase. As already pointed out trivalents must segregate unequally or leave one chromosome in the equator, whereas univalents segregate at late anaphase in an entirely irregular manner. Thus only a small proportion of first anaphase (and consequently spore) nuclei by chance receive a genetically balanced chromosome complement of the monoploid or near-diploid number. Normally such spores are the only viable ones. Following the pairing and chiasma formation or univalent segregation spore nuclei may contain balanced combinations of

(I) Chromosomes derived from opposite parents of the triploid.

(II) Cross over chromosomes which combine parts of semi-homologous chromosomes derived from opposite

parents.

Such spores if they function in fertilising the original diploid or tetraploid will be the means of introducing genes from the diploid into the tetraploid and vice versa.

Amongst the diploid plantain types meiotic behaviour of the hybrids C29 (PsS9 x P84) and C28 (P. carinata x PsS9) indicates a high degree of chromosomal homology and a high potentiality of gene exchange between the three parental types.

Similarly in the tetraploids the behaviour of the hybrid C22 gives no indication of any cytological barrier to gene exchange within the group.

The triploids examined resulted from the tetraploid P. alpina (Edin.) crossed by various diploids. The tetraploids C37 N.S. and C38 N.S. originated from open pollination of the triploids C37 and C38 respectively. Meiosis in these triploids showed that the chromosomes of the tetraploid parent were sufficiently homologous to pair and form chiasmata with chromosomes of the diploids P81, P84 and P91. The derivative tetraploids must contain twelve chromosomes from the triploid parent and twelve from the pollen grains of unknown origin. Vegetatively the triploids are vigorous, but, as was only to be

expected from the observed chromosome distributions at anaphase they were almost completely sterile. On the other hand the few tetraploid plants produced by these triploids were fertile, not only when crossed among themselves but also when crossed with another tetraploid of wild origin. It is to be expected that in nature tetraploids of this kind are most likely to arise in habitats occupied by a mixed population of diploids and tetraploids. If then even a single triploid happened by chance to become established and leave tetraploid progeny the requisite opportunities for the fertilisation of such offspring by tetraploids would be available.

A further means of gene transfer from diploid to tetraploid populations arises in the production of aberrant spores by the diploid. Diploid pollen grains have been recorded, for example, in Primula sinensis, Darlington (1931a), P. floribunda, Upcott (1939), Brassica japonica, Fukushima (1930), Allium schoenoprasum, Levan (1936), Aquilegia, Skalinska (1939). Such diploid pollen will readily fertilise a related tetraploid giving rise to a tetraploid zygote deriving half its gene complement from the diploid, half from the tetraploid parent. Probably such gene transfer will not usually attain

great proportions, although it seems definite that with samples of mixed monoploid and diploid pollen grains the diploid pollen is more successful in fertilising the tetraploid - a result in keeping with the general behaviour of incompatibility.

Such production of diploid gametes by members of the diploid sea plantain population is advanced as a method whereby the tetraploid population can acquire genes present in the diploid population. For example the tetraploid hybrid, C21, obtained from a cross P. alpina (Edin.) tetraploid x PsS9 diploid was studied both at mitosis and meiosis, and it seems that the functional pollen from the diploid parent must have carried twelve, instead of the normal six, chromosomes. Meiosis in PsS9 proved to be perfectly regular and there were no grounds for supposing that failure of reduction was likely to occur. Tetrad formation was also found to be regular. Although tetraploid pollen mother cells were not observed in PsS9 a single case was seen in C29, a hybrid between PsS9 diploid and P84 diploid, of a pollen mother cell which had approximately twenty-four chromosomes and which had probably arisen from a tetraploid archesporial cell. From such tetraploid mother cells, by analogy with the behaviour of other matings, a

proportion of diploid spores is to be expected in nature. In contrast to the above example a fertile tetraploid C46 arose from the cross P113 diploid and P65 tetraploid, a fact which strongly suggests that in the wild exchange of genes could take place as a result of the production of unreduced egg nuclei.

While no experimental evidence has so far been obtained concerning the origin of tetraploids directly from diploids it has been possible to demonstrate how the variability of the former can be enriched in regions where the two populations are contiguous. In nature the production of triploids is likely to be negligible except perhaps where self-incompatible tetraploids occur in very low frequency among diploids, or vice versa. However, the chances of triploids ever establishing themselves in greater concentration than as sporadically occurring individuals is extremely improbable. Thus in the absence of an intermediate triploid population the line of demarkation between the cytologically stable diploid and tetraploid populations will remain clearly defined. Notwithstanding this lack of an appreciable connecting population the absorption of diploid population genes by the tetraploids can still take place directly and unobtrusively through the agency

of diploid gametes. As mentioned in the introduction these two populations were provisionally regarded as belonging to different coenospecies, i.e. their status as independent units was recorded as complete. But in view of the evidence now available this experimental taxonomic conception is no longer tenable and the true population status of both diploids and tetraploids is more adequately expressed, in terms of taxonomy, by regarding them as separate ecospecies within a coenospecies. In other words the diploid and tetraploid populations constitute two unquestionable units of evolutionary significance having a low potential capacity for the exchange of genes.

V. SUMMARY.

(1) The basic chromosome number in the group of plantains allied to Plantago maritima L. is x = 6. The N. American and N. European populations are 2x; those inhabiting the European Alpine region are of two kinds (a) 2x and (b) 4x.

(2) Chromosome complements of all 2x forms show a high degree of homology and similarly all 4x types. In 2x and 4x plants the average chiasma frequency is in no case significantly different from 1.00 chiasma per bivalent or potential bivalent. Both 2x and 4x plants have a similar frequency (circa 9.6%) of bivalents with two chiasmata. Only P. carinata (2x) appears to differ in this respect.

(3) The meiotic behaviour of 3x and 4x hybrids and hybrids between 4x and 2x plants strongly suggest that the 4x parental types are autotetraploid.

(4) The 4x, like the 2x populations form a cytologically stable and intrafertile group. Their fertility is attributed to a low chiasma frequency and a consequently low incidence of multivalents.

(5) The occasional acquisition by the 4x population of genes present in the 2x population has been demonstrated as follows:-

(a) The fertilisation of a 4x egg by a 2x pollen grain from a 2x plant.

(b) The fertilisation of a 2x egg nucleus of a 2x plant by a normal 2x gamete of a 4x plant, and

(c) The production of 4x zygotes from 3x hybrids.

(6) The occurrence of a certain amount of chromosome inversion has been inferred from the behaviour of hybrid material. The possible limitation of gene exchange by inversion is discussed.

(7) Since both 2x and 4x populations lead an almost independent evolutionary existence in consequence of their potentially low capacity for gene interchange it is suggested that, taxonomically, they should be regarded as separate ecospecies within a common coenospecies.

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